Peroxisome proliferator-activated receptors (PPARs) and mitochondria in relation to lifestyle-related diseases

Therese Halvorsen Røst

Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

April 2008

SCIENTIFIC ENVIRONMENT

This work was performed at the Lipid Research Group, Section of Medical Biochemistry, Institute of Medicine, the University of Bergen.

The work was funded by a PhD-grant from the Western Norway Regional Health Authority in the period of 2004–2007.

ACKNOWLEDGEMENTS

This work was performed at Section for Medical Biochemistry, Institute of Medicine, the University of Bergen, with financial support from the Western Norway Regional Health Authority. A financial donation was also received from the Novo Nordisk Foundation and Oddrun Mjålands Stiftelse for Kreftforskning.

Professor Rolf Kristian Berge has been my supervisor. I thank him for his optimism, enthusiasm and eagerness, and for giving me the opportunity to evolve as a scientist through participation in and interaction with national and international scientific environments.

I would also like to thank my co-supervisor, Professor Gunnar Mellgren, for his help and support, and for introducing me to the world of nuclear receptors and coactivators.

I am grateful to Kari H. Mortensen, Kari Williams, Liv Kristine Øysæd, Randi Sandvik, Randi Solheim and Svein Krüger for skilful technical assistance. To them and to all my other present and previous colleagues; Endre Dyrøy, Hege Wergedahl, Ingunn A. Wergeland, Jon Skorve, Karl Johan Tronstad, Kjetil Berge, Marte Aanestad, Oddrun A. Gudbrandsen, Pavol Bohov, Randi Rogde and Ziad Muna, I am grateful for all help, valuable discussions and a good working environment. Especially, my thanks go to Kjetil, Karl Johan, Hege and Oddrun for always answering my many questions. Kjetil and Karl Johan have been my go-to guys in regards to cell work. Kjetil has always been a great travelling companion on the many conferences all over the world, and Karl Johan has been of great help in clarifying many aspects of mitochondrial function. Hege and Oddrun have also been very helpful in clarifying the many pathways of lipid metabolism, and I really appreciate the friendly atmosphere we have in our office, together with Marte, including the breaks of chatting over cake and hot chocolate.

All co-authors are acknowledged for their valuable contributions to the papers of this thesis. To all the other people with whom I am surrounded by every day at work, I am thankful for nice lunch breaks and a pleasant and stimulating environment.

My sincere thanks go to my parents and stepparents, my mother and Lennart in particular, my brother Martin, my sister Jeanette, the rest of my family, my “in-laws” and my friends for always being supportive, encouraging and very proud of me.

Last, but not least, I am grateful to my boyfriend Erlend for his love, support and faith in me. I am also very thankful for his patience and his will for taking care of the household and having dinner ready during the most hectic months of this work.

Bergen, April 2008

Therese Halvorsen Røst

CONTENTS

ABBREVIATIONS

AADA	Arylacetamide deacetylase
ACO	Acyl-coenzyme A oxidase
ACS	Acyl-coenzyme A synthetase
AF	Activation function
apo	Apoprotein
BMI	Body mass index (kg/m^2)
BSA	Bovine serum albumin
CoA	Coenzyme A
CPT	Carnitine palmitoyltransferase
CVD	Cardiovascular disease
DBD	DNA binding domain
HDL	High density lipoprotein
HMG	3-hydroxy-3-methylglutaryl
HRE	Hormone response element
IDL	Intermediate density lipoprotein
LBD	Ligand binding domain
LDL	Low density lipoprotein
LXXLL	Protein motif where L is the amino acid leucine, and X is any amino acid
NAFLD	Non-alcoholic fatty liver disease
NR	Nuclear receptor
PGC-1	Peroxisome proliferator-activated receptor gamma coactivator-1
PPAR	Peroxisome proliferator-activated receptor
PPRE	Peroxisome proliferator-activated receptor response element
RT-PCR	Reverse transcriptase polymerase chain reaction
RXR	Retinoid X receptor
SRC	Steroid receptor coactivator
T2DM	Type 2 diabetes mellitus
TNFα	Tumour necrosis factor-α
TSA	Tetradecylselenoacetic acid
TTA	Tetradecylthioacetic acid
UCP	Uncoupling protein
VLDL	Very low density lipoprotein

SUMMARY

Obesity is a growing life-style related health problem, and is often accompanied by dyslipidemia, insulin resistance and inflammation. This may in turn lead to hyperinsulinemia, increased risk of non-alcoholic fatty liver disease, type 2 diabetes mellitus and cardiovascular disease. Together, these abnormalities have been clustered into the metabolic syndrome.

The present thesis focuses on two modified fatty acids, tetradecylthioacetic acid (TTA) and tetradecylselenoacetic acid (TSA), with a biochemical and mechanistic angle to the description and possible therapeutic options of obesity related disorders and risk factors of the metabolic syndrome and cardiovascular disease. As major regulators of lipid metabolism and their involvement in glucose metabolism and inflammation, the peroxisome proliferator-activated receptors (PPARs; PPARα, PPARδ, PPARγ) have been in focus in these studies.

We have shown that through increased fatty acid oxidation, TTA may prevent the development of fatty liver induced by tamoxifen, a drug commonly used in patients with breast cancer. Furthermore, TTA had lipid lowering effects in patients with type 2 diabetes mellitus, through mechanisms seeming to involve PPARα and PPARδ activation, and increased mitochondrial fatty acid oxidation. The role of PPARα in TTA mediated effects was investigated in PPARα deficient mice, and revealed that TTA was able to stimulate fatty acid oxidation in mice lacking PPARα, indicating that PPARα is involved in but not essential for the lipid lowering effects of TTA. In cell culture PPAR activation studies we confirmed that TTA may stimulate the activity of all PPARs. Moreover, we found that PPARγ coactivator (PGC)-1 is a potent PPAR coactivator, and that TTA further induced the PGC-1 mediated coactivation of PPARδ. Thus, it seems reasonable to believe that the activity of both PPARα and PPARδ, with a substantial contribution of nuclear receptor coactivators, PGC-1 in special, may be conductive to TTA's mechanism of action.

Studies with TSA suggest that it exert potent antioxidant, antiinflammatory and hypolipidemic properties, potentially involving PPAR-related mechanisms. Antioxidants protect against oxidative stress and inflammation, which, in combination with hyperlipidemia, are important mediators of atherogenesis. Based on this, it is tempting to hypothesize that TSA could be an interesting antiatherogenic approach to atherosclerotic disorders.

Altogether the present thesis demonstrates that TTA and TSA may have a possible therapeutic potential in obesity related disorders and risk factors of the metabolic syndrome and cardiovascular disease. Their mechanism of action seems to include the action of PPARs, with contribution of nuclear receptor coactivators.

LIST OF PAPERS

Paper I

Causes and prevention of tamoxifen-induced accumulation of triacylglycerol in rat liver

O. A. Gudbrandsen*, T. H. Rost*, and R. K. Berge.

Journal of Lipid Research 2006; 47: 2223–2232.

Paper II

Tetradecylthioacetic acid attenuates dyslipidemia in type 2 diabetic patients, possibly by dual PPARα/δ activation and increased mitochondrial fatty acid oxidation

Kristian Løvås, Therese H. Røst, Jon Skorve, Rune J. Ulvik, Oddrun A. Gudbrandsen, Pavol Bohov, Andreas J. Wensaas, Arild C. Rustan, Rolf K. Berge, Eystein S. Husebye.

Submitted to *Diabetologia*, April 2008.

Paper III

The pan-PPAR ligand, tetradecylthioacetic acid, induces hepatic fatty acid oxidation in PPARα-/- mice possibly through stimulation of PGC-1 dependent coactivation of PPARδ

Therese H. Røst, Line L.H. Moi, Kjetil Berge, Bart Staels, Gunnar Mellgren, Rolf K. Berge

Manuscript.

Paper IV

Tetradecylselenoacetic Acid, a PPAR Ligand with Antioxidant, Antiinflammatory, and Hypolipidemic Properties

Endre Dyrøy*, Therese H. Røst*, Reidar J. Pettersen, Bente Halvorsen, Oddrun A. Gudbrandsen, Thor Ueland, Ziad Muna, Fredrik Müller, Jan E. Nordrehaug, Pål Aukrust, Rolf K. Berge.

Arteriosclerosis, Thrombosis, and Vascular Biology 2007;27:628-634.

* The authors contributed equally to this work

INTRODUCTION

This thesis presents a biochemical and mechanistic angle to the description and possible therapeutic options of some of the factors related to obesity and the metabolic syndrome. This introduction will present the relevant disorders, followed by a more detailed introduction to the molecular participants that may play a role in regulation of these disorders and thus being potential therapeutic targets.

Obesity and the metabolic syndrome

Obesity is created by a positive energy balance, which is when energy intake exceeds energy utilization, and is a growing life-style related health problem. Obesity is defined as body mass index (BMI) >30 kg/m^2, and overweight as 25 kg/m^2 < BMI < 30 kg/m^2. In many countries in Europe, more that 50% of the population is defined as being overweight, while up to 30% are defined as being obese (1).

The metabolic abnormalities that often accompany obesity include dyslipidemia, hypertension, impaired glucose tolerance, insulin resistance and inflammation. These abnormalities may in turn lead to hyperinsulinemia and an increased risk of non-alcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (2). Together, these abnormalities have been clustered into the metabolic syndrome. The World Health Organisation defines the metabolic syndrome as the coincidence of two or more of the following features; central obesity, elevated level of plasma triacylglycerols, hypertension, microalbuminuria, impaired glucose regulation or insulin resistance (3). Thus, the metabolic syndrome may be regarded as an obesity- and lifestyle-related disease.

Obesity and inflammation

Metabolism and the inflammatory response are integrated processes which under normal conditions are beneficial for the maintenance of good health, but under conditions of metabolic challenge this interaction can be damaging. Obesity is characterised by a state of chronic low-level inflammation in the adipose tissue, with an accumulation of macrophages and an upregulation of a variety of inflammatory and stress-response genes (4).

The link between obesity and inflammation was first established by Hotamisligil et al. who demonstrated a positive correlation between adipose tissue mass and the expression of the proinflammatory tumour necrosis factor-α (TNFα) (5). Later, the adipose tissue has been established as a major secretory organ, secreting several different proteins, collectively termed

adipokines. Adipokines linked to the inflammatory response include cytokines (e.g. TNFα, interleukins) and acute phase proteins (e.g. C-reactive protein, fibrinogen) (6).

Insulin resistance and type 2 diabetes mellitus

To protect the body against hypoglycaemia during fasting and against a too high glucose level after a high-carbohydrate meal, homeostatic mechanisms are in place to maintain blood glucose levels within a very narrow range. This is achieved mainly through gluconeogenesis/glucose production by the liver and through peripheral glucose uptake by skeletal muscle, heart muscle and adipose tissue.

Insulin resistance and T2DM are associated with visceral obesity and large amounts of adipose tissue. As fat accumulates in adipose tissue there is an ongoing increase in the levels of plasma fatty acids, leading to increased fatty acid exposure to non-adipose tissues. The fatty acids may induce insulin resistance in muscle by inhibiting insulin-dependent glucose uptake and intracellular glucose utilization (7) as well as by altering insulin signalling and thereby reducing insulin-stimulated glucose transport (8). The fatty acids also stimulate hepatic overproduction of glucose (9). Pro-inflammatory adipokines from adipose tissue also interfere with insulin signalling pathways and contribute to the development of insulin resistance and T2DM (4).

The risk of CVD is increased in patients with T2DM (10, 11). Therefore, it is recommended that traditional CVD risk factors, dyslipidemia being the most important, is treated and carefully monitored in patients with T2DM (12).

Atherosclerosis and cardiovascular disease

Atherosclerosis is a progressive process responsible for diseases of the heart and blood vessels, collectively termed CVDs. Several risk factors are associated with the development of atherosclerosis and CVD, and they coincide with the disorders related to obesity and the metabolic syndrome: hyperlipidemia, high low density lipoprotein (LDL)-cholesterol levels, low high density lipoprotein (HDL)-cholesterol levels, T2DM, hypertension, inflammation and oxidative stress (13, 14).

The atherosclerotic process is initiated by infiltration and accumulation of LDL-cholesterol within the arterial wall. This results in oxidation of LDL particles, endothelial alterations, inflammatory response and accumulation of cholesterol within macrophages. The presence of these cholesterol-rich macrophages defines the earliest pathological lesion, referred to as fatty streak (14). Fatty streaks are not occlusive and cause no overt symptoms,

but may progress into more advanced and complicated lesions called atherosclerotic plaque. The atherosclerotic plaque consists of foam cells, necrotic cells and accumulating amounts of lipids, surrounded by smooth-muscle cells and a fibrous cap. Activated immune cells accumulate within the lesion and induce a more severe inflammatory response that reduces the stability of the plaque and may lead to rupture and occlusion of the artery, heart attack and stroke (13, 14).

Non-alcoholic fatty liver disease

Fatty liver is considered as the hepatic expression of the metabolic syndrome (15). The development of fatty liver is a consequence of fatty acid accumulation in the hepatocytes, and causative factors include hyperlipidemia, obesity and T2DM (16, 17). At the time of diagnosis, most patients with NAFLD have few or no symptoms of liver disease, and moderate increase in serum aminotransferases is the most common and often the only laboratory abnormality found in these patients (18). NAFLD include a wide spectrum of liver injuries, which may proceed from uncomplicated fat accumulation to more severe injury with inflammation and loss of liver function, or hepatocellular carcinoma.

Contributory mechanisms that have been suggested for NAFLD include insulin resistance, and an imbalance between the enzyme systems that regulate the uptake, synthesis, oxidation and transport of fatty acids (19), leading to increased accumulation of fatty acids within the hepatocytes.

Unfortunately, drug-induced fatty liver also occurs, and represents a challenge to the pharmaceutical industry and for the physicians. Tamoxifen is an example of a drug used in treatment of breast cancer that may lead to fatty liver and visceral fat accumulation (20, 21).

Modified fatty acids

Fatty acids are carboxylic acids with hydrocarbon chains that are saturated, mono- or polyunsaturated. The length and degree of saturation of the hydrocarbon chain largely determine the properties of the fatty acids and compounds of which they are a part.

Almost 20 years ago, a group of modified fatty acids were developed in order to test a hypothesis related to peroxisome proliferation (22). This was the start of the still ongoing expedition revealing and exploring the pleiotropic effects of tetradecylthioacetic acid (TTA; C14-S-acetic acid). TTA is a modified fatty acid with a sulphur atom inserted in 3-position in the carbon backbone (Figure 1). The chemical properties of TTA are similar to natural saturated fatty acids with comparable chain length, however, the metabolism and metabolic

effects of TTA are distinctive. TTA is converted to CoA thioesters like natural fatty acids, it can also be delta-9 desaturated (23) and elongated (24), but due to the sulphur atom TTA cannot be β-oxidised and are instead sulphur- and ω-oxidised to small dicarboxylic acids (25).

As a ligand for the peroxisome proliferator-activated receptors (PPARs) (26, 27) TTA stimulate the expression of several PPAR target genes, which regulate several aspects of lipid and glucose metabolism. The pleiotropic effects of TTA include increased mitochondrial proliferation and fatty acid oxidation as well as reduced plasma lipid levels, ultimately leading to reduced obesity and improved insulin sensitivity (25). Additionally, TTA is demonstrated to inhibit LDL-oxidation and lipid peroxidation (25, 28), and to have antiinflammatory properties through regulation of cytokine release (25, 29).

Another modified fatty acid, tetradecylselenoacetic acid (TSA; C14-Se-acetic acid), was developed in an attempt to find a stronger antioxidant than TTA (28). Selenium is a stronger reducing agent than sulphur, therefore TSA was synthesized by inserting a selenium atom in the 3-position of the carbon backbone (Figure 1). As with TTA, this modification makes TSA resistant to fatty acid β-oxidation. TSA is far less studied that TTA, but as hypothesised, the stronger antioxidant properties of TSA was confirmed in studies performed in vitro (28).

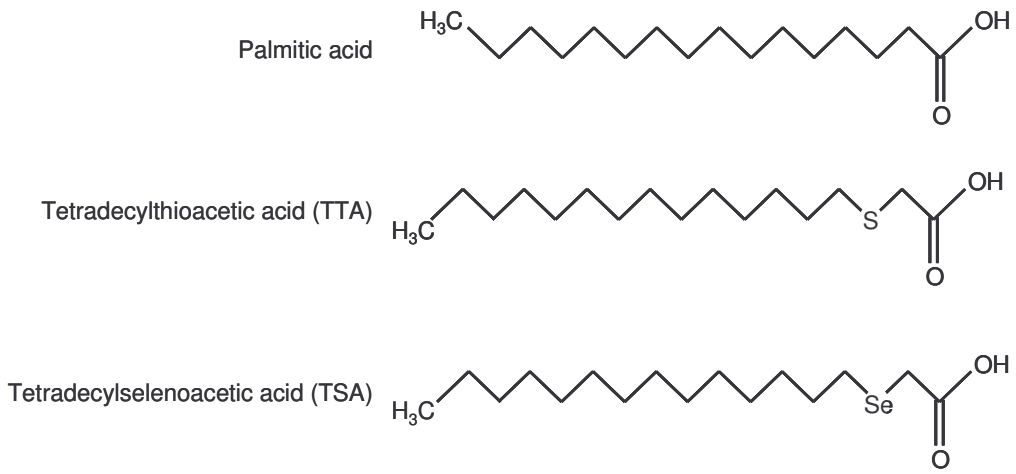

Figure 1: Structure of one natural and two modified fatty acids.

Lipid metabolism

Uptake and transport

Lipids belong to a chemically diverse group of fatty acid containing compounds, such as phospholipids and triacylglycerols, with diverse functions such as maintaining membrane integrity and fluidity, and serving as metabolic fuel to provide energy. Lipids also play crucial roles as regulators of cellular functions by acting as ligands, enzyme coactivators, cell membrane-anchors or intracellular messenger molecules.

Dietary fatty acids are usually esterified as triacylglycerols. In the intestine, the action of water-soluble lipases converts triacylglycerols into free fatty acids, which diffuse into the intestinal epithelial cells before they are reconverted to triacylglycerol and incorporated with cholesterol and apoproteins (apo) to form lipoproteins called chylomicrons. Lipoproteins are spherical particles containing a hydrophobic lipid core and a hydrophilic protein surface. Lipoproteins are classified according to their density, which is defined by their various combinations of lipids and proteins.

Through the blood stream chylomicrons are transported to liver, adipose tissue, skeletal muscle and the heart. Lipoprotein lipase hydrolyses the triacylglycerols to free fatty acids, which are taken up in the target tissues where they are either oxidised to provide energy, or reesterified for storage as triacylglycerols. The remnant chylomicrons are subsequently taken up by the liver (Figure 2). Very low density lipoproteins (VLDL) are produced in the liver and secreted into the blood stream for transport to extrahepatic tissues, where lipoprotein lipase in the capillaries releases free fatty acids from the VLDL triacylglycerols. The loss of triacylglycerols converts VLDL to smaller remnant VLDL, or intermediate density lipoproteins (IDL). Some of the IDL is taken up in the liver, through LDL receptor, or remains in the blood stream where it undergoes further triacylglycerol hydrolysis, resulting in cholesterol rich LDL. LDL is taken up in liver and extrahepatic tissues through binding to LDL receptor (Figure 2). Cholesterol is transported from extrahepatic tissues to the liver in HDL, in a process called reverse cholesterol transport. Nascent HDL is produced in the liver or the intestine and contains very little cholesterol. In the process of forming a mature spherical HDL, cholesterol is transported from extrahepatic tissues and into the nascent HDL. Lecithin-cholesterol acyltransferase esterifies free cholesterol in the nascent HDL and converts it into a mature HDL. HDL-cholesterol is delivered to the liver and may be secreted in the bile (Figure 2).

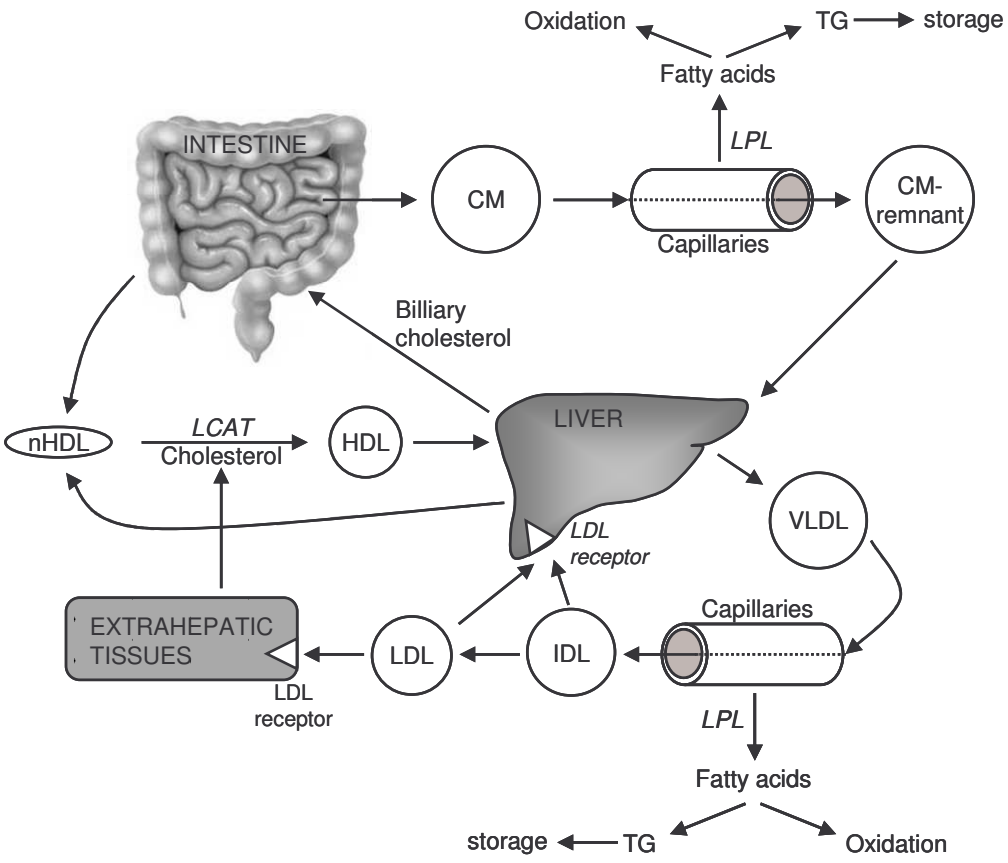

Figure 2: Schematic overview of the main pathways for lipoprotein mediated lipid transport.

CM, chylomicron; IDL, intermediate density lipoprotein; LCAT, Lecithin-cholesterol acyltransferase; LDL, low density lipoprotein; LPL, lipoprotein lipase; (n)HDL, (nascent) high density lipoprotein; TG, triacylglycerol; VLDL, very low density lipoprotein.

Before fatty acids that are delivered by the lipoproteins can be utilized by the cells, they need to be transported over the plasma membrane. As of today, two modes of cellular uptake of fatty acids are presented; passive diffusion over the plasma membrane, and protein-mediated transport. In recent years there has been more focus on the protein-mediated transport, and the process of diffusion over the plasma membrane has been considered quantitatively less important (30). The fatty acid transporters that have been identified include the plasma membrane fatty acid binding protein (31), fatty acid translocase/CD36 (32-34), and a group of 6 fatty acid transport proteins that are expressed in a tissue specific manner and show different transport capacities (35-37). These proteins may all act alone or in combination with each other in manners that may differ in various tissues (30). Although the physical mechanisms of the protein-mediated fatty acid transport are not yet fully elucidated, the process is considered as highly regulated (30).

Once inside the cell, the fatty acids are transported to other sub-cellular compartments by the cytoplasmic fatty acid binding protein (38). The hydrophilic cytoplasmic environment is not suitable for diffusion of fatty acids, and the fatty acids are therefore embedded in the hydrophobic core of the fatty acid binding protein, which then facilitate the intracellular transport between membranes (39). A common target for the intracellular fatty acid transport is the mitochondria, which is the major site for fatty acid β-oxidation, although β-oxidation also occurs in peroxisomes.

Activation and Oxidation

Before mitochondrial fatty acid oxidation the fatty acids must be activated by the attachment of coenzyme A (CoA) through an energy-rich thioester bond with the fatty acid carboxyl group. The esterification is mediated by acyl-CoA synthetase (ACS), an enzyme present in many cells and which exists in several variants with different fatty acid specificities (40). Long chain fatty acids are activated by long chain ACS in the outer mitochondrial membrane before they can be transported over the mitochondrial membrane by the activities of carnitine palmitoyltransferase (CPT)-1 and CPT–2 (Figure 3). CPT-1 in the outer mitochondrial membrane facilitates the exchange of the fatty acyl group from CoA to carnitine and is frequently described as the rate limiting step of mitochondrial β-oxidation (41). Thereby the fatty acyl-carnitine crosses the inner mitochondrial membrane via the carnitine/acylcarnitine translocase. On the inside of the inner membrane, CPT-2 facilitates the formation of fatty acyl-CoA by the exchange of carnitine with CoA (Figure 3). The transport of short and medium chain fatty acids over the mitochondrial membranes occurs independently of the carnitine system, and they are activated to acyl-CoAs by short- and medium-chain ACS in the mitochondrial matrix (42) (Figure 3).

Once inside the mitochondrial matrix, the fatty acyl-CoA may undergo β-oxidation. In each subsequent β-oxidation cycle, the fatty acyl-CoA is shortened by two carbons, yielding acetyl-CoA and a chain shortened fatty acyl-CoA (Figure 3). In the case of odd-chained fatty acids, one mole of propionyl-CoA is produced for each mole odd-chained fatty acids. A complete oxidation of acetyl-CoA to CO_2 occurs via the citric acid cycle together with donation of electrons to the respiratory chain and ATP production.

Transport of fatty acids into peroxisomes is independent of carnitine, and the fatty acids seem to be transported over the peroxisomal membrane as acyl-CoAs. The peroxisomal β-oxidation process is similar to the mitochondrial process (Figure 3), although the enzymes are not the same (43). Acyl-CoA oxidase (ACO) regulates the first and rate-

limiting step of peroxisomal β-oxidation, which is considered as a chain-shortening and partial degradation pathway for very long chain fatty acids. Due to the lack of a citric acid cycle, the complete degradation to CO_2 can not occur in the peroxisomes (44). Chain shortened fatty acids are rather exported out of the peroxisomes to the mitochondria for further oxidation (44) (Figure 3).

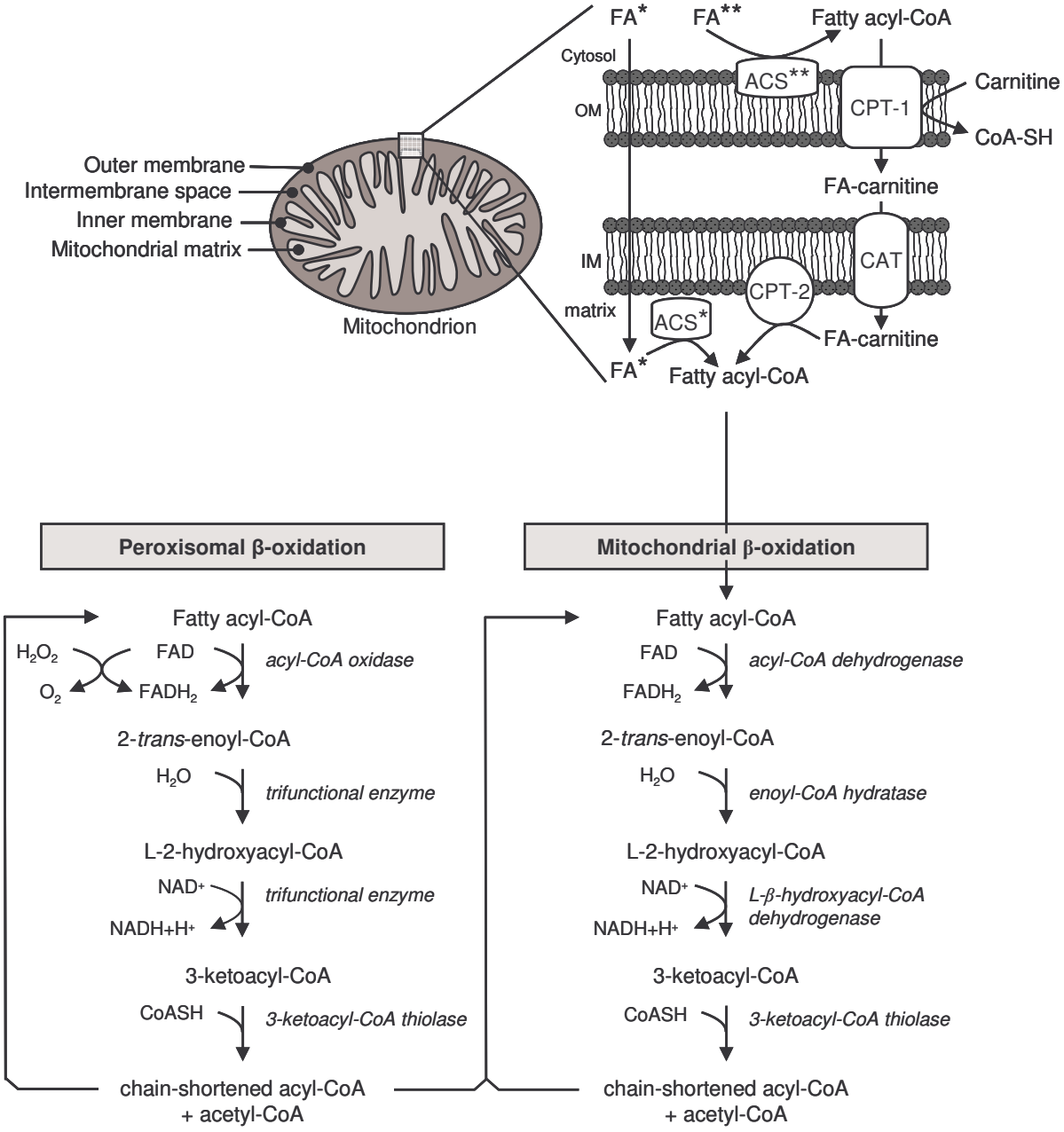

Figure 3: Mitochondrial and peroxisomal fatty acid β-oxidation.

* Short and medium chain fatty acids, as well as ** long chain fatty acids cross the mitochondrial membrane for β-oxidation. Very long chain fatty acids are chain-shortened in the peroxisomes and exported to the mitochondria for complete degradation. ACS; acyl-CoA synthetase; CAT, carnitine/acylcarnitine translocase; CoA, coenzyme A; CPT-1/2, carnitine palmitoyltransferase-1/2; FA, fatty acid ; IM, inner membrane; OM, outer membrane.

The nuclear receptor superfamily

Nuclear receptors (NRs) comprise a big superfamily of ligand-activated transcription factors that stimulate the expression of target genes involved in metabolism, development and reproduction. The NRs are activated by a great variety of ligands, they show a variable degree of ligand specificity and one receptor may interact with several different ligands. Receptors for which there are yet no identified ligands are called orphan receptors.

The NR family members share a similar domain structure. The domains are designated A-F from the N-terminal region to the C-terminal (Figure 4). The A/B domain region is not well conserved among family members. This region modulates the receptor's promoter- and cell-specific activity, and the difference between receptor subtypes normally lies in this region. The A/B region of many receptors harbours an activation function (AF)-1 domain through which transcriptional activity is regulated ligand-independently. Region C harbours the DNA binding domain (DBD) which is the most conserved domain of the NR superfamily. It confers the ability to activate the transcription of target genes by binding specifically to hormone response elements (HREs) in their promoter region. The variable D region serves as a hinge between the DBD and the ligand binding domain (LBD), allowing rotation of the DBD. This rotation is essential in receptor conformational changes associated with ligand binding. The LBD, region E/F, is also highly conserved in the NR superfamily, but still differs enough to allow specific ligand recognition. Structure analyses have demonstrated a conserved structure, formed by 12 α-helices, that form a cavity which functions as a ligand binding pocket (45). The LBD binds ligands with high specificity, and performs several functions regulated by ligand binding. These functions include interaction with heat-shock proteins, homo- and hetero-dimerisation and transcriptional activity. Binding of antagonists may block these functions. The AF-2 domain is present in the C-terminal end of the LBD, and is responsible for ligand-dependent transcriptional activation (46).

Based on evolutionary analysis of the conserved domains, the NRs have been divided into six different subfamilies (47) and later given a unified nomenclature (48), Table 1. Some receptors are further divided into subtypes which may differ in terms of ligand preference, tissue distribution and/or target genes of which they regulate transcription.

Table 1: The 6 subfamilies/classes of the nuclear receptor superfamily, their abbreviation and subtypes, unified nomenclature, selection of ligands and major field of function.

Class	Name	Abbreviation	Nomenclature	Ligand	Major field of function
1	Thyroid hormone receptor	TRα, β	NR1A1, 2	Thyroid hormone	Thyroid hormone response in differentiation, growth, metabolism, physiology
	Retinoic acid receptor	RARα, β, γ	NR1B1, 2, 3	Retinoic acid	Embryonic development
	Peroxisome proliferator-activated receptor	PPARα PPARδ/β PPARγ	NR1C1 NR1C2 NR1C3	Fatty acids, fibrates, WY14.643 Fatty acids, L165.041 Fatty acids, prostaglandins, thiazolidinediones, BRL49653	Lipid and glucose metabolism
	Reverse erbA	Rev-erbα, β	NR1D1, 2	Orphan	Cell proliferation and physiology
	RAR-related orphan receptor	RORα RORβ, γ	NR1F1 NR1F2, 3	Cholesterol, cholesteryl sulphate Retinoic acid	Cell survival in the central nervous system
	Liver X receptor	LXRα, β	NR1H1, 2	Oxysterols	Cholesterol homeostasis
	Farnesoid X receptor	FXRα FXRβ	NR1H4 NR1H4	Bile acids, fexaramine Lanosterol	Bile acid homeostasis
	Vitamin D receptor	VDR	NR1I1	1-25(OH)2 vitamin D3	Calcium homeostasis, bone development and mineralization, cell growth and differentiation
2	Hepatocyte nuclear factor 4	HNF4α HNF4γ	NR2A1 NR2A2	Fatty acyl-CoA thioesters Orphan	Liver development
	Retinoid X receptor	RXRα, β, γ	NR2B1, 2, 3	9-cis-retinoic acid	Embryonic patterning, organogenesis, differentiation, heterodimer partner for other NRs
	Testis receptor	TR2 TR4	NR2C1 NR2C2	Orphan Orphan	Negative modulator of other nuclear receptors
	Tailless	TLL	NR2E2	Orphan	Neuronal development
	Chicken ovalbumin upstream promoter-transcription factor	COUP-TFI, II	NR2F1, 2	Orphan	Peripheral nervous system development, angiogenesis, vascular remodelling, heart development
3	Estrogen receptor	ERα ERβ	NR3A1 NR3A2	Estradiol, tamoxifen, raloxifene Estradiol	Maintenance of the reproductive, cardiovascular, musculoskeletal and central nervous systems
	Estrogen-related receptor	ERRα ERRβ, γ	NR3B1 NR3B2, 3	Orphan Diethylstilbestrol, 4-OH tamoxifen	Lipid metabolism
	Glucocorticoid receptor	GR	NR3C1	Glucororticoids	Development, metabolism, immune response
	Mineralcorticoid receptor	MR	NR3C2	Aldosterone, spirolactone	Electrolyte and fluid balance in the kidney
	Progesterone receptor	PR	NR3C3	Progesterone	Reproduction
	Androgen receptor	AR	NR3C4	Testosterone	Development and function of male reproductive organs
4	Nerve growth factor-induced factor B	NGFI-B	NR4A1	Orphan	Stress stimuli response, apoptotic stimuli, signalling in the hypothalamic-pituitary axis.
5	Steroidogenic factor 1	SF-1	NR5A1	Orphan	Mammalian sexual development, differentiation of steroidogenic tissues
	Liver receptor homologous protein 1	LRH1	NR5A2	Orphan	Steroid, bile acid and cholesterol metabolism
6	Germ cell nuclear factor	GCNF	NR6A1	Orphan	Transcriptional repressor, vertebrate embryogenesis

In order to promote transcription, NRs bind through their DBD to HREs in the promoter region of their target genes. Two HRE consensus sequences have been identified. The consensus sequence AGAACA is mainly recognised by steroid receptors (class 3; Table 1), whereas AGG/TTCA serves as a consensus recognition motif for the remaining NRs (49). Some receptors bind as monomers to HREs, but most receptors bind as homo- or heterodimers to HREs composed of two core motifs. The arrangement and spacing between the motifs are determinants in conferring selectivity and specificity. The dimeric HRE half-sites can be configured as palindromes (→←), inverted palindromes (←→) or direct repeats (→→) (reviewed in (49)).

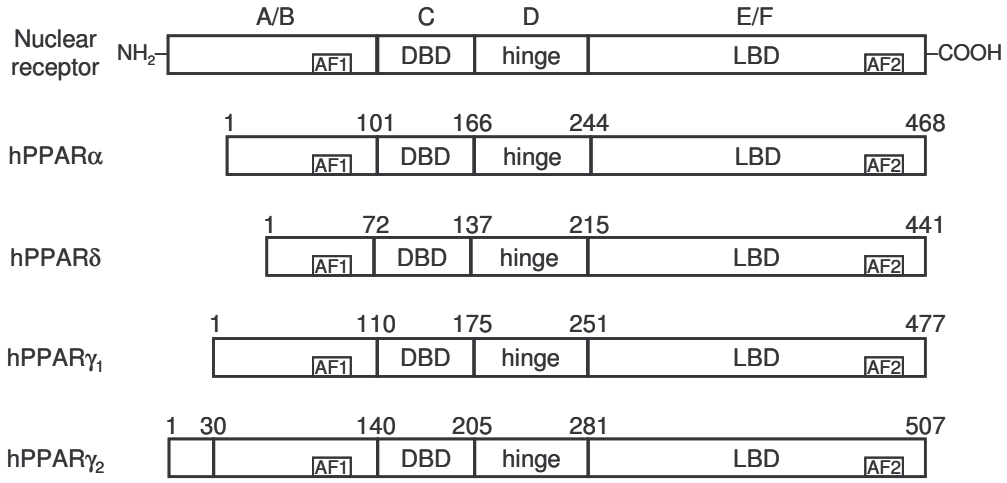

Figure 4: The structural and functional domains of a general nuclear receptor and of PPARs.

A-F denotes the different NR domain regions. The numbers indicate amino acid residues. AF1/2, activation function 1/2; DBD, DNA binding domain; LBD, ligand binding domain; hPPAR, human peroxisome proliferator-activated receptor.

The peroxisome proliferator-activated receptors; PPARα, PPARδ, PPARγ

The PPARs are class 1 members of the NR superfamily, Table 1. Three PPAR subtypes have been identified, PPARα (50-52), PPARδ (also called PPARβ) (51, 53, 54) and PPARγ (51, 54, 55). Three different PPARγ mRNA exist, which are produced by differential use of three promoters and alternative splicing of three 5' exons. However, the translation of PPARγ_1 and PPARγ_3 mRNA result in the same protein (56), while PPARγ_2 mRNA codes for a protein with 30 extra amino acids in the N-terminal region. The two PPARγ isoforms are however

demonstrated to share similar response to several different PPARγ specific ligands (57, 58). As common for the NR family members, the PPARs also have a modular structure consisting of several functional domains, where the LBD and DBD are well conserved (Figure 4).

Tissue distribution

The PPAR expression level varies in different tissues, and in some tissues the PPARs are coexpressed. PPARα is highly expressed in metabolically active tissues, including liver, heart, kidney, skeletal muscle, brown fat and large intestine. PPARγ$_1$ and γ$_2$ are abundant in adipose tissue and present at lower levels in skeletal muscle. In addition, PPARγ$_1$ is found in liver, macrophages and heart. PPARδ is expressed in a wide range of tissues, with relatively higher levels in brain, adipose tissue and skin (58, 59).

PPAR function

The PPARs stimulate the transcription of a great variety of genes, including those involved in almost all aspects of lipid metabolism, glucose and energy balance, inflammation, differentiation and proliferation (Table E), as reviewed in (59-62). The presence of a functional PPAR response element (PPRE) in the promoter region of the gene classifies it as a PPAR target gene. However, some genes could also be classified as PPAR dependent, as their gene-regulation is reported to be dependent upon PPAR, although a functional PPRE in their promoter region is not yet identified (60).

The PPAR system is complex, and in addition to having their own specified function and set of target genes, their function, tissue distribution and range of target genes may overlap (Table 2). However, there are several ways by which the PPAR tissue and target gene specificity are regulated. First of all, a differential tissue distribution controls tissue specific PPAR action. In tissues where PPARs are coexpressed, PPAR subtype specific ligands contribute to control PPAR subtype specificity. Also, the PPRE sequence, including the 5'-flanking extension, confer a level of PPAR subtype specific control (63).

Table 2: PPAR function in inflammation, lipid and energy metabolism in selected tissues.

	PPARα (61, 64)	**PPARδ** (62, 65, 66)	**PPARγ** (59, 64)
Liver 	FA oxidation ↑ FA uptake ↑ HDL lipoprotein ↑ Inflammation ↓ VLDL production ↓	FA synthesis ↑ Glc output ↓	
Skeletal muscle 	FA oxidation ↑ FA uptake ↑ Glc intolerance/insulin resistance ↑ TG lipolysis ↑ Glc utilization ↓	FA oxidation ↑ FA transport ↑ Mitochondrial respiration ↑ Thermogenesis ↑ Slow-twitch fibres ↑	
White adipose tissue 		FA oxidation ↑ Thermogenesis ↑ FA transport ↑	Differentiation ↑ Lipid storage ↑
Brown adipose tissue 		FA oxidation ↑ FA transport ↑ Thermogenesis ↑	
Artery / Blood vessel 	Reverse cholesterol transport ↑ Inflammation ↓	HDL cholesterol ↑ Inflammation ↓	Inflammation ↓

FA, fatty acid; Glc, glucose; HDL, high density lipoprotein; TG, triacylglycerol; VLDL, very low density lipoprotein

PPAR ligands

PPARs are defined as generous hosts, capable of specifically interacting with more than one ligand. The PPAR subtype specific ligand preference is achieved through the difference between their LBDs.

Naturally occurring PPAR ligands include fatty acids and fatty acid derived compounds (Table 3). Synthetic ligands are also identified (Table 3), and some of them have been used in treatment of diabetes or hyperlipidemia. Several lipid-lowering agents, including fibrates and WY14.643, are classified as PPARα ligands (54, 67). The anti diabetic thiazolidinediones, such as rosiglitazone/BRL49653, are characterised to be PPARγ ligands (68, 69). In a series of novel compounds, L165.041 turned out to be a selective PPARδ agonist with hypolipidemic effects (70, 71).

PPARs form a permissive heterodimer with retinoid X receptor (RXR), which means that either partner can regulate the transcriptional activity of the DNA-bound complex by interacting with its agonist, on its own or when both partners are ligand-bound. RXR is the receptor for 9-*cis*-retinoic acid (72).

In addition to ligand-dependent activation, phosphorylation by the various kinases also affects PPAR activity in both ligand–dependent and –independent manners that depend on the PPAR subtype and cellular context, reviewed in (73).

Table 3: List of selected endogenous and synthetic PPAR ligands.

	PPARα	**PPARδ**	**PPARγ**
Endogenous ligands	USFAs (54, 67, 74-77)	USFAs (54, 74, 75, 77)	USFAs (54, 74, 76, 77)
	SFAs (75)	SFAs (75)	9- and 13-HODE (78)
	8(S)-HETE (74-76)	8(S)-HETE (77)	15d-PGJ_2 (78-80)
Synthetic ligands	Fenofibrate (81)	GW501516 (82)	BRL49653 (68, 69)
	Clofibrate (67, 75)	L165.041(70)	Pioglitazone (68, 83)
	Cipofibrate (67, 75)	TTA (26, 27)	Ciglitazone (68, 83)
	Gemfibrozil (67, 75)		TTA (26, 27)
	WY14.643 (54, 67, 75, 77)		
	TTA (26, 27, 75)		

BRL49653, Rosiglitazone; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; 15d-PG J_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; SFAs, saturated fatty acids; TTA, tetradecylthioacetic acid; USFAs, unsaturated fatty acids

DNA binding properties

For DNA binding, the PPARs depend on RXR as a hetero-dimer binding partner. They bind to a PPRE, which is an imperfect direct repeat of two core recognition motifs spaced by one nucleotide (DR-1 motif). A consensus PPRE sequence has been given as 5′-AGGTCA A AGGTCA-3′ (84). PPARs bind to the upstream extended core hexamer of the DR-1, whereas RXR occupies the downstream motif (85). This consensus sequence is common for the other class 1 members of the NR superfamily, but the spacing between the core recognition motifs (AGGTCA) may vary and thereby contribute to receptor specific recognition of the response element. In contrast to the RXRα homodimer which also binds to the DR-1 motif, the PPARα/RXRα heterodimer was demonstrated to be highly dependent on a 5'-flanking extension sequence (C A/G A/G A A/T CT) (86). The presence of this 5' sequence favoured

DNA binding of the heterodimer compared to the homodimer. Also PPAR subtype-specific PPRE recognition is mainly dependent on this 5'-flanking extension, and PPARα and PPARδ seem to be more dependent on this extension sequence than on a perfect DR-1 PPRE sequence as compared to PPARγ (63).

Nuclear receptor coactivators

The activity and function of PPARs and other NRs are strongly dependent on the interaction with coregulator proteins, which includes both corepressors and coactivators. These proteins associate with NRs, and while corepressors attenuate the NR transcriptional activity, coactivators, which will be in focus in this thesis, promote their transcriptional activity.

The coactivators represent a diverse group of proteins, but common for most of them is their NR interacting domain, containing LXXLL α-helical motifs, where L represents the amino acid leucine, and X is any amino acid (87). The stabilisation of the AF-2 helical domain in activated NRs creates a charge clamp and a hydrophobic pocket in the LBD, which define the coactivator interaction domain (88). The charge clamp is made from two conserved charged amino acid residues (lysine and glutamate) in the LBD that positions the LXXLL motif so that the leucine residues may pack into the intervening hydrophobic pocket.

While the charge clamp and amino acid residues in the hydrophobic pocket are conserved among the NRs, several NRs still show specific preferences towards different coactivators. This may be explained by the ligand dependent conformational changes in the AF-2 domain of the NR. But also, the number of LXXLL motifs, as well as their length and orientation relative to each other, varies among coactivators (87), and may account for some of the specificity. Additionally, the LXXLL motifs are divided into four classes, depending on their flanking amino acids (89, 90), and different NRs have preferences for different classes of LXXLL motifs.

The coactivators are also diverse in means of their function; they posess diverse enzymatic activities that contribute to transcriptional activation. Through histone acyltransferase, methyltransferase and chromatin remodelling activities, coactivators may relax the tightly packed chromatin structure and enhance the accessibility of other transcription factors to allow genes to be transcribed. Through their splicing control function, coactivators are also involved in splicing (maturation, removal of introns) of the newly transcribed gene. Additionally, coactivators may function as signal integrators, transferring

cell-surface signals further into the cell. These aspects are reviewed by D.M. Lonard and B.W. O'Malley (91).

PPAR interaction with coactivators

The unliganded PPAR/RXR heterodimer interacts with corepressors that suppress the transcriptional activity. In the unliganded state the receptors are proposed to exist in a conformational equilibrium where the LBD structure is variable. Activation by ligand binding releases the corepressors and stabilises the AF-2 helical domain in a conformation that is permissive for coactivator interaction (88, 92, 93). The coactivators can actuate or enhance NR activity through remodelling of the histone and chromatin structure and/or bridging to the basal transcription machinery (91). Several different coactivators have been described, that are able to interact with the PPARs (94). The coactivators are expressed in a tissue specific manner and their expression level may be influenced by physiological status, which again may contribute to the tissue-specific PPAR activity. Additionally, structure analyses and other in vitro assays have suggested that the PPAR structure is altered in a ligand-specific way, resulting in distinct interactions with coactivators, allowing distinct ligand-specific downstream effects (88, 92, 93, 95-97).

Of PPAR interacting coactivators relevant for this thesis, are two coactivators from the steroid receptor coactivator (SRC) family, SRC-1 and SRC-2, as well as PPARγ coactivator (PGC)-1α (termed PGC-1 hereafter) from the PGC-1 family. These coactivators will be more closely presented in the following sections.

The steroid receptor coactivators

The steroid receptor coactivator family (SRC), comprise three members; SRC-1 (98), SRC-2 (also called TIF2 or GRIP1) (99, 100) and SRC-3 (also called AIB1, pCIP or ACTR) (101-104), which are all widely expressed in many cell types and tissues. The members of the SRC family share a common domain structure, with a conserved N-terminal domain for DNA interaction and interaction with other coactivators. Centrally located are three copies of the conserved LXXLL motifs, through which they interact with ligand activated NRs. At the C-terminal these receptors have a transcriptional activation function. In this region the SRCs interact with other coactivators through another set of LXXLL motifs (105). Through these interactions the SRC coactivators becomes part of multiunit complexes consisting of proteins with the capacity of modifying histones, remodelling chromatin and interacting with the basal

transcription machinery (102, 106, 107). Additionally, a weak histone acetyl transferase function is present in the C-terminal end of SRC-1 (108) and SRC-3 (102).

Several studies have demonstrated the ability of SRC-1 (88, 95, 109-111) and SRC-2 (95, 110-112) to interact with and to coactivate all the different PPARs. In 1998 Nolte et al. (88) showed the crystal structure of SRC-1 bound to PPARγ, showing the coactivator interaction domain and the charge clamp, which is believed to be essential for most NR/coactivator interactions. The interactions between PPARs and coactivators have been demonstrated to be both dependent and independent of exogenous ligands.

The biological function of the SRCs includes roles in development, fertility and hormone response. In addition, studies have revealed a role of SRC-1, SRC-2 and SRC-3 in energy homeostasis and adipogenesis (113, 114), as depicted in Table 4. These studies show that while SRC-1 -/- mice have reduced energy expenditure and tend to be obese, the mice lacking SRC-2 have increased adaptive thermogenesis and are protected against high fat diet induced obesity (114). Studies of SRC-3 demonstrate an essential role of this coactivator in adipocyte differentiation in that it regulates several genes involved in the differentiation process (113).

Table 4: The role of coactivators in energy metabolism and mitochondrial function in selected tissues.

	SRC-1 (114)	**SRC-2** (114)	**SRC-3** (113, 115)	**PGC-1** (116-120)
Liver 	FA oxidation ↑	FA oxidation ↓		FA oxidation ↑ Gluconeogenesis ↑
Muscle 				Mitochondrial biogenesis ↑ Oxidative phosphorylation ↑ Thermogenesis ↑ FA oxidation ↑
White adipose tissue 	Thermogenesis ↓	Adipogenesis ↑	Adipogenesis ↑	
Brown adipose tissue 	Thermogenesis ↑	Thermogenesis ↓	Thermogenesis ↓	Mitochondrial biogenesis ↑ Oxidative phosphorylation ↑ Thermogenesis ↑

FA, fatty acid; SRC, steroid receptor coactivator; PGC-1, peroxisome proliferator-activated receptor γ coactivator-1

PPARγcoactivator-1

Another family of coactivators is the PGC-1 gene family. The family consists of PGC-1 (116), the close homolog PGC-1β (121) and PGC-1-related coactivator (122). The domain structure of these coactivators is not homologous to that of the SRCs. PGC-1 contains a N-terminal activation domain, harbouring a LXXLL motif, as well as a C-terminal RNA binding domain and two serine and arginine rich regions. In addition PGC-1 has three consensus protein kinase A phosphorylation sites (116). The two other PGC-1 gene family members have a similar domain structure.

PGC-1 does not have an intrinsic histone acetyl transferase activity, but interacts through its N-terminal activation domain with other coactivators that harbours such activity (123). Through its C-terminal RNA binding domain and serine/arginine rich regions PGC-1 is also found to form complex with the preinitiation and elongation forms of RNA polymerase II, as well as other elongation factors. Thus, PGC-1 function as a modulator of transcription initiation, elongation and mRNA processing of its target genes (124).

PGC-1 is shown to be a transcriptional coactivator of many NRs, including the PPARs (116, 125, 126) and nuclear respiratory factors 1 and 2 (117), serving as a pleiotropic regulator of multiple pathways of cellular metabolism. Although there are similarities between the nature of PPAR interaction with PGC-1 compared to the interaction with SRC-1 and SRC-2, there are also some differences. Both ligand dependent and independent interactions are observed, and PGC-1 also interacts through its LXXLL domain with the AF-2 domain of PPARα and PPARδ (125, 126). However, weaker interactions have also been observed in the central hinge region of PPARα (126). In addition, PGC-1 interaction with PPARγ is demonstrated to be through a proline rich region of PGC-1 and the central hinge region or the DNA binding domain of PPARγ (116).

When PGC-1 was identified as a PPARγ interacting protein in brown fat cells (116), it revealed a coactivator with major functions in energy homeostasis, Table 4. PGC-1 is expressed in brown fat, heart, kidney and brain. Upon cold exposure the mRNA expression of PGC-1 is induced in skeletal muscle, and the expression level in brown fat is increased (116). PGC-1 expression is induced in liver upon fasting, and in the liver of mice with diabetes, both insulin-deficient and insulin-resistant (120). In brown adipose tissue and skeletal muscle PGC-1 stimulates mitochondrial DNA production and induces mitochondrial respiration through increased expression of genes of the respiratory chain. In brown adipose tissue, mitochondrial uncoupling and thermogenesis are promoted through the induction of

uncoupling protein-1 (116), and mitochondrial uncoupling through uncoupling protein (UCP)-2 is demonstrated in skeletal muscle (117). Through interaction with the PPARs, PGC-1 stimulates the expression of genes involved in fatty acid oxidation in both muscle (118) and liver (119). Additionally, PGC-1 is demonstrated to control hepatic gluconeogenesis (120).

Interaction between mitochondria, PPARs and coactivators

The mitochondria are considered as the main cellular transducers of metabolic energy because they contain enzyme systems for fatty acid β-oxidation, the citric acid cycle, ketogenesis, oxidative phosphorylation and thermogenesis. The mitochondrial function is closely connected to the activity of PPARs as several genes for proteins involved in mitochondrial function are considered as PPAR targets. These include the genes for proteins regulating fatty acid activation (ACS), mitochondrial fatty acid uptake (CPT-1 and CPT-2), β-oxidation (acyl-CoA dehydrogenase), ketogenesis (3-hydroxy-3-methylglutaryl (HMG)-CoA synthase), respiration and thermogenesis (cytochrome C, cytochrome oxidase, UCP-1 and UCP-2) (59, 61, 62). By functioning as PPAR coactivators, SRC-1, SRC-2 and PGC-1 participate in linking the PPARs to mitochondrial function. In addition, especially PGC-1 may also play an independent role in stimulation of oxidative phosphorylation, mitochondrial thermogenesis and mitochondrial biogenesis (116, 117). The gene expression of PGC-1 is also reported to be induced by PPARδ activation (127). Thus, the function of mitochondria, PPARs and coactivators are interconnected and constitute important pathways relevant for regulation of obesity and obesity related disorders.

AIMS OF THE PRESENT STUDY

The present thesis focuses on two modified fatty acids, TTA and TSA, with a biochemical and mechanistic angle to the description and possible therapeutic options of obesity related disorders and risk factors of the metabolic syndrome and cardiovascular disease. As major regulators of lipid metabolism and their involvement in glucose metabolism and inflammation, the PPARs have been in focus in these studies.

Specific aims of this study have been to:

- Elucidate the mechanisms behind tamoxifen-induced fatty liver, and evaluate the potential of TTA to prevent this development in combinatory treatment with tamoxifen.

- Investigate the hypolipidemic effects of TTA in a clinical study in patients with type 2 diabetes mellitus, and to consider the role of PPARs and mitochondrial fatty acid oxidation in these effects.

- Evaluate the role of PPARα in mediating the hypolipidemic effects of TTA, and to study the role of PPAR coactivators in TTA-dependent and -independent PPAR activation.

- Evaluate the potential antioxidant, antiinflammatory and hypolipidemic properties of TSA and to consider the role of PPARs and mitochondrial fatty acid oxidation in these effects.

EXPERIMENTAL MODELS

In experimental science considerations are always made in regards to finding model systems that allow the design of efficient and effective methods that make it possible to pinpoint the relevant questions and to extrapolate the results into the relevant context. In the present studies, several different model systems have been employed, including several in vitro cell models, in vivo animal models and a phase II clinical study in patients with T2DM.

Cell cultures

The ability to culture cells in vitro provides a valuable technique for studying living cells under controlled conditions. Cell lines originating from various tissues and species are commercially available and have to a great extent contributed to our understanding of molecular interactions between proteins and regulatory mechanisms in metabolism. In the present investigation various cell culture models were used with respect to relevant tissues or to feasibility and simplicity of certain methods.

*Human HepG2 liver and MCF-7 breast cancer cells; transfection and gene expression studies (**Papers II, III and IV**)*

HepG2 cells are human liver cancer cells. It is a well established cell line that is easy to handle and to propagate, and widely used in basic scientific research. Although it is a cancer cell line and therefore harbours certain dissimilarities to primary human liver cells (cf. www.atcc.org), HepG2 cells are intended to be a model for liver tissue in the present studies. As the availability of primary human liver cells is rather limited and their propagation in cell cultures not straight forward, HepG2 cells have functioned as an easy-access and simple model with a stable phenotype for in vitro analyses.

The MCF-7 cells represents a human breast cancer cell line, which in the same way as HepG2 cells are easy to handle and to propagate, and widely used in basic scientific research.

Transfection assays, where we have evaluated PPAR activation by TTA or TSA, have been involved in all of the papers including the HepG2 and MCF-7 cell lines. Not all cell cultures are suitable for the transfection procedure, but optimization procedures demonstrated that these cell lines were easy to transfect, and therefore they were natural choices for use in our transfection assays. Gene expressions by real time reverse transcriptase polymerase chain reaction (RT-PCR) have also been measured in these cells. The MCF-7 cells were only

employed in one study (**Paper III**) for comparison with HepG2 cells and the detection of cell type specific effects.

*Human skin fibroblasts and murine J774 macrophages; uptake of oxidised LDL (**Paper IV**)*
Human skin fibroblasts were obtained from biopsies of healthy individuals and since they mainly express receptors for native LDL and not oxidised LDL, they were employed in studies of radiolabelled LDL oxidation.

Likewise, due to their high capacity of oxidised LDL uptake, and limited amount of native LDL receptors, the murine J774 macrophages were useful for measuring oxidised LDL uptake. An advantage of these cells is that they differentiate easily into mature macrophages without the need of any specific chemical induction.

Radiolabelled LDL particles were oxidised in a cell free system in the presence or absence of TSA. The fibroblasts and macrophages made it possible to evaluate the degree of LDL oxidation by measuring the uptake of either oxidised or native radiolabelled LDL.

*Human peripheral blood mononuclear cells; cytokine release (**Paper IV**)*
Human peripheral blood mononuclear cells were isolated from blood samples from healthy blood donors, providing the ability to study processes in a controlled environment with relevance to the human situation. An immune response was stimulated by the addition of phytohemagglutinin, lipopolysaccaride or TNFα, which are all commonly used agents for such a purpose. The way TSA treatment affected the release of proinflammatory and antiinflammatory cytokines was evaluated.

*Human skeletal muscle cells; mitochondrial fatty acid oxidation (**Paper II**)*
Human skeletal muscle cells were obtained from a previously established cell-bank of satellite cells from human muscle biopsies of healthy donors (128). The cells were employed in studies for mitochondrial fatty acid β-oxidation and real time RT-PCR studies of fatty acid oxidation enzymes, where the effect of TTA was evaluated. As mentioned before, the use of primary cells derived from humans allows for the opportunity to measure several parameters in a controlled environment but still keeping the studies relevant for the human situation. The results from these cells were used as a supplement to the clinical study of patients with T2DM receiving TTA, in attempt to elucidate the TTA's mechanism of action.

Animal-models

In comparison to in vitro models, in vivo animal models represent an advantage in that it is possible to study whole body metabolism and to evaluate interaction and crosstalk between different tissues. All experiments with animal-models in this thesis (**Papers I, III and IV**) were conducted according to the Guidelines for the Care and Use of Experimental Animals.

*Sprague-Dawley rats; tamoxifen-induced fatty liver and cotreatment with TTA (**Paper I**)*

Tamoxifen is commonly used as an effective treatment for oestrogen receptor positive breast cancer (129), but is unfortunately associated with an increased risk of developing fatty liver (20, 21) through mechanism that are not yet fully elucidated. The hypolipidemic effects of TTA (25) make it a potential strategy in prevention of tamoxifen-induced fatty liver.

In order to study the effect of tamoxifen on lipid metabolism in the liver in more detail, and to study the potential of TTA to prevent tamoxifen-induced fatty liver, we performed a study in female Sprague-Dawley rats from Taconic M&B A/S (Ry, Denmark). This is a traditional animal model used in virtually all disciplines of biomedical research. In order to resemble breast cancer patients, the rats were treated with a single dose of 20 mg of 7,12-dimethylbenz[a]anthracene (DMBA; D-3254; Sigma-Aldrich Norway AS, Oslo, Norway) to induce cancer.

*Wistar rats; the antioxidant, antiinflammatory and hypolipidemic potential of TSA (**Paper IV**)*

In previous studies TSA has been demonstrated as a stronger antioxidant than TTA (28). We wanted to investigate if TSA also had antiinflammatory and hypolipidemic properties. As a complement to in vitro model systems for a model with higher relevance to the human situation, we employed another traditional and widely used rat model; male Wistar rats (Mol:Wist) from Møllegård Breeding Laboratorium (Ejby, Denmark).

*Domestic pigs; the potential of TSA to inhibit restenosis after percutaneous coronary intervention (**Paper IV**)*

Percutaneous coronary intervention is a therapeutic procedure to treat arteries that are narrowed due to the build-up of atherosclerotic plaque, and involves the inflation of a balloon within the coronary artery to crush the plaque into the walls of the artery. Restenosis is a common problem after this intervention, and the process of restenosis resembles the atherosclerotic process. Thus, studying restenosis after percutaneous coronary intervention provides an applicable model of the atherosclerotic process.

As the heart physiology of pigs is very similar to human heart physiology, this is an animal model with high relevance to humans in matters concerning the heart and the cardiovascular system.

Highly qualified heart surgeons performed percutaneous coronary intervention in the arteries of healthy domestic pigs. Arterial injury was applied using an oversized semi compliant angioplasty balloon, and TSA was administered at the site of artery injury. This procedure allowed us to study the effect of TSA on processes that are linked to the development of atherosclerosis.

*PPARα-/- mice; the role of PPARα in hypolipidemic mechanisms mediated by TTA (**Paper III**)*

Genetic mutation models open an opportunity to obtain in depth knowledge about the functions of single targeted proteins in vivo. In order to get a better understanding of the specific role of PPARα in TTA mediated effect on lipid metabolism, we performed a study in PPARα-/- mice on a Sv/129 genetic background (130), with sv/129 mice as wild type control. The PPARα-/- mice have a targeted disruption of the PPARα gene, and are demonstrated to lack PPARα protein expression (130). The increased expression of PPARα target genes in response to PPARα ligands, as observed in wild type mice, is also absent in the PPARα-/- mice (130). Thus, these mice function as models to study the effect of PPARα deficiency.

Open-label clinical study

Due to the promising observations of TTA in regard to preventing risk factors of the metabolic syndrome in animal studies, we wanted to investigate if TTA was well tolerated also in humans, and if we could observe similar effects to those observed in animals. This first study performed with TTA in human patients was conducted with an open-labelled design (**Paper II**). This was an exploratory study of TTA in human patients, with safety as a major issue. Patients with T2DM received a daily morning dose of 1g TTA for 28 days (**Paper II**). Termination of other treatments (lipid lowering or hypoglycaemic drugs) was chosen to eliminate the risk of TTA/drug interactions. The study was performed in accordance with the Declaration of Helsinki and consistent with Good Medical Practise and applicable regulatory requirements.

SUMMARY OF THE PRESENT INVESTIGATION

Paper I

Causes and prevention of tamoxifen-induced accumulation of triacylglycerol in rat liver.

O. A. Gudbrandsen*, T. H. Rost*, and R. K. Berge.

Journal of Lipid Research 2006; 47: 2223–2232.

* equal contribution

Objectives:

Tamoxifen can induce hepatic steatosis in women. In this study, we wanted to elucidate the mechanism behind the tamoxifen-induced accumulation of triacylglycerol in liver in female rats, and we hoped to prevent this development by combination treatment with the modified fatty acid Tetradecylthioacetic acid (TTA).

Results:

We found that the increased hepatic triacylglycerol level after tamoxifen treatment was accompanied by decreased acetyl-coenzyme A carboxylase (ACC) and FAS activities, increased glycerol-3-phosphate acyltransferase (GPAT) activity, and a tendency to increased diacylglycerol acyltransferase (DGAT) activity. The activities and/or mRNA levels of enzymes involved in hepatic β-oxidation, ketogenesis, and uptake of lipids were unaffected by tamoxifen, whereas the uptake of fatty acids was decreased. Combination treatment with tamoxifen and TTA (Tam+TTA) normalized the hepatic triacylglycerol level and increased the activities of ACC, FAS, GPAT, and DGAT compared with tamoxifen treated rats. The activities and mRNA levels of enzymes involved in β-oxidation, ketogenesis, and uptake of lipids were increased after Tam+TTA treatment.

Conclusions:

In conclusion, tamoxifen increased the hepatic triacylglycerol level, probably as a result of increased triacylglycerol biosynthesis combined with unchanged β-oxidation. The tamoxifen-induced accumulation of triacylglycerol was prevented by cotreatment with TTA, through mechanisms of increased mitochondrial and peroxisomal β-oxidation.

Paper II

Tetradecylthioacetic acid attenuates dyslipidemia in type 2 diabetic patients, possibly by dual PPARα/δ activation and increased mitochondrial fatty acid oxidation

Kristian Løvås, Therese H. Røst, Jon Skorve, Rune J. Ulvik, Oddrun A. Gudbrandsen, Pavol Bohov, Andreas J. Wensaas, Arild C. Rustan, Rolf K. Berge, Eystein S. Husebye.

Submitted to *Diabetologia*, April 2008.

Objectives:

The modified fatty acid, tetradecylthioacetic acid (TTA), is previously demonstrated to improve transport and utilization of lipids, and to increase mitochondrial fatty acid oxidation in animal and cell studies. The aim of the present study was to determine whether TTA treatment of patients with type 2 diabetes mellitus and dyslipidemia could improve lipoprotein profile and possibly blood glucose. Moreover, by performing in vitro experiments, we aimed to study the role of peroxisome proliferator-activated receptors (PPARs) in TTA's mechanism of action.

Results:

Sixteen male type 2 diabetic patients received 1g TTA daily for 28 days in an open-labelled study, with measurement of parameters of lipid metabolism, glucose metabolism and safety. The mechanism of action was further investigated in a human liver cell line (HepG2) and in cultured human skeletal muscle cells (myotubes). Mean LDL cholesterol level declined from 4.2 to 3.7 mmol/L (P = 0.0004), accompanied by increased levels of the HDL apo A1 and apo A2, and a decline in LDL/HDL ratio. Total fatty acid levels declined significantly, especially the polyunsaturated n-3 fatty acids docosahexaenoic acid (-13%, P = 0.001) and eicosapentaenoic acid (EPA) (-10%, P = 0.033). Glucose metabolism was not altered and the drug was well tolerated. In cultured liver cells TTA acted as a pan-PPAR agonist, with predominant PPARα and PPARδ activation at low TTA concentrations. In cultured skeletal muscle cells, TTA and a PPARδ agonist, but not the PPARα or PPARγ agonists, increased the mitochondrial fatty acid oxidation.

Conclusions:

We demonstrate for the first time that TTA attenuates dyslipidemia in type 2 diabetic patients. The results suggest that these effects may occur through mechanisms involving PPARα and PPARδ activation, resulting in increased mitochondrial fatty acid oxidation.

Paper III

The pan-PPAR ligand, tetradecylthioacetic acid, induces hepatic fatty acid oxidation in PPARα-/- mice possibly through stimulation of PGC-1 dependent coactivation of PPARδ

Therese H. Røst, Line L.H. Moi, Kjetil Berge, Bart Staels, Gunnar Mellgren, Rolf K. Berge

Manuscript

Objectives:

Tetradecylthioacetic acid (TTA) is a hypolipidemic modified fatty acid, and ligand for peroxisome proliferator-activated receptor (PPAR)α, PPARδ and PPARγ. The mechanisms of TTA-mediated effects seem to involve the action of the PPARs, but the effects have not yet been assigned to any specific PPAR subtype. In PPARα-/- mice we wanted to study the role of PPARα after TTA treatment. We also performed in vitro experiments in order to obtain mechanistic knowledge of how TTA affected PPAR activation in the presence of the PPARγ coactivator (PGC)-1 and the steroid receptor coactivator (SRC)-1 and SRC-2, which have been associated with energy balance and mitochondrial biogenesis.

Results:

We show that TTA stimulates fatty acid β-oxidation in PPARα-/- mice. In HepG2 and MCF-7 cells TTA acts as a pan-PPAR ligand, and we demonstrate that PGC-1, SRC-1 and SRC-2 have cell type and PPAR-specific effects in concert with TTA. In absence of exogenous ligands, SRC-1 did not induce PPAR activity. SRC-2 stimulated PPAR activity especially in MCF-7 cells, while PGC-1 was a far more potent PPAR coactivator in both cell lines. When the coactivators were overexpressed, pronounced effects of TTA were observed especially for PPARδ and PPARγ, but TTA further induced the PGC-1 dependent coactivation of only PPARδ.

Conclusions:

We conclude that PPARα is involved in, but not required for the hypolipidemic mechanisms of TTA. It appears that the activity of PPARδ, with a substantial contribution of nuclear receptor coactivators, PGC-1 in special, is conductive to TTA's mechanism of action.

Paper IV

Tetradecylselenoacetic Acid, a PPAR Ligand With Antioxidant, Antiinflammatory, and Hypolipidemic Properties

Endre Dyrøy*, Therese H. Røst*, Reidar J. Pettersen, Bente Halvorsen, Oddrun A. Gudbrandsen, Thor Ueland, Ziad Muna, Fredrik Müller, Jan E. Nordrehaug, Pål Aukrust, Rolf K. Berge

* equal contribution

Arteriosclerosis, Thrombosis, and Vascular Biology 2007;27:628-634.

Objectives:

Antioxidants protect against oxidative stress and inflammation, which, in combination with hyperlipidemia, are important mediators of atherogenesis. Here we present a selenium-substituted fatty acid, tetradecylselenoacetic acid (TSA). The objective of the present study was to evaluate the antioxidant, antiinflammatory and hypolipidemic properties of TSA, and to consider the role of PPARs and mitochondrial fatty acid oxidation in these effects.

Results:

We show that TSA exerts antioxidant properties by delaying the onset of oxidation of human low density lipoprotein (LDL), by reducing the uptake of oxidized LDL in murine macrophages and by increasing the mRNA level of superoxide dismutase in rat liver. TSA also showed antiinflammatory effects by suppressing the release of interleukin-2 and -4, and increasing the release of interleukin-10 in human blood leukocytes. In addition, TSA decreased the plasma triacylglycerol level and increased the mitochondrial fatty acid β-oxidation in rat liver. In pigs, TSA seemed to reduce coronary artery intimal thickening after percutaneous coronary intervention. In HepG2 cells TSA activated all peroxisome proliferator-activated receptors (PPARs) in a dose-dependent manner.

Conclusions:

Our data suggest that TSA exert potent antioxidant, antiinflammatory and hypolipidemic properties, potentially involving PPAR-related mechanisms. Based on these effects, it is tempting to hypothesize that TSA could be an interesting antiatherogenic approach to atherosclerotic disorders.

GENERAL DISCUSSION

Since life-style related disorders and the metabolic syndrome are increasingly prevalent in both children and adults in many parts of the world, extensive research is being conducted in hope to restrain and prevent this development. Common for these disorders are visceral obesity and dyslipidemia, lipid modulating agents are therefore considered to have a promising potential in treatment strategies.

The present thesis focus on two modified fatty acids, TTA and TSA, with a biochemical and mechanistic angle to the description and possible therapeutic options of some of the factors related to obesity and the metabolic syndrome. As major regulators of lipid metabolism and their involvement in glucose metabolism and inflammation, the PPARs have been in focus in these studies.

The potential of TTA in disorders related to obesity and the metabolic syndrome

Since TTA was developed in order to test a hypothesis related to peroxisome proliferation almost 20 years ago (22), this modified fatty acid has been proven as highly bioactive with pleiotropic effects, and especially as a potent regulator of lipid metabolism (25). Several cell culture and animal studies have revealed that through mitochondrial proliferation and increased catabolism of fatty acids, as well as through antiproliferative, antioxidant and antiinflammatory properties, TTA may have a major impact on disorders related to obesity and the metabolic syndrome (25).

Tamoxifen-induced fatty liver and potential of combinatory treatment with TTA

Tamoxifen is a widely used drug against breast cancer. An undesirable side-effect is, however, that tamoxifen may induce fatty liver and visceral fat accumulation (20, 21). Fatty liver and visceral obesity are associated with other components of the metabolic syndrome, and is a problem that needs attention. The mechanism of tamoxifen-induced fatty liver is not fully elucidated, but we (**Paper I**) and others (131, 132) have tried to come closer to an explanatory mechanism.

We found that treatment with tamoxifen for 14 days in rats significantly increased hepatic triacylglycerols and serum transaminases which are indicators of liver damage (**Paper I**). The increased hepatic triacylglycerol level may be caused by increased biosynthesis, impaired fatty acid β-oxidation, increased hepatic uptake, or reduced secretion of lipids from the liver, or by a combination of two or more of these factors. As also reported by others (131,

132), we found that the fatty acid synthase, and thus fatty acid biosynthesis, was decreased by tamoxifen treatment (**Paper I**). However, it seemed like the biosynthesis of triacylglycerol was increased in the liver after tamoxifen treatment (**Paper I**), this was accompanied with unchanged β-oxidation and may explain the hepatic accumulation of triacylglycerols.

Others have reported the decreased expression fatty acid synthase, with a consequent increase in malonyl-CoA which inhibits CPT-1 and fatty acid oxidation, as the primary cause of tamoxifen-induced fatty liver (132). Another group also demonstrated that tamoxifen was able to inhibit mitochondrial fatty acid β-oxidation and CPT-1 activity (131). The discrepancy between these (131, 132) and our (**Paper I**) results may be explained by different experimental designs, including the choice of mice versus rats, female or male, healthy or cancerous, and probably most importantly the administered tamoxifen dose and length of treatment.

The hepatic uptake of triacylglycerol seemed to be unaffected by tamoxifen treatment, as assessed by gene expression of LDL and VLDL receptors (**Paper I**). The increased liver triacylglycerol combined with reduced serum triacylglycerol, may suggest that the secretion of triacylglycerol from the liver was reduced, which has previously been observed after tamoxifen treatment (131). The secretion of triacylglycerol-rich VLDL was not measured directly, but the unchanged expression of apo B and arylacetamide deacetylase (AADA), which play important roles in VLDL assembly (133), indicated that hepatic VLDL secretion was not affected. However, increased secretion of VLDL has been observed by others without any change in AADA mRNA level (133) and post-translational regulation of apo B is of importance (134). Thus, direct measurement of VLDL secretion is needed to get a clear impression of the VLDL secretion after tamoxifen treatment. Importantly, tamoxifen is also demonstrated to accumulate in liver mitochondria, where it impairs mitochondrial respiration and mitochondrial DNA synthesis (131, 135). In addition to the possible mechanisms of reduced hepatic triacylglycerol secretion, decreased mitochondrial β-oxidation and more severe impairment of mitochondrial function may be the mechanism of tamoxifen-induced fatty liver.

In order to investigate if TTA could prevent tamoxifen-induced fatty liver, we treated rats with a combination of tamoxifen and TTA (**Paper I**). The tamoxifen-induced accumulation of hepatic triacylglycerol was prevented by cotreatment with TTA, although the hepatic lipid biosynthesis and uptake of lipids from the circulation seemed to be increased (**Paper I**). However, in comparison to rats receiving tamoxifen, those that received tamoxifen in combination with TTA showed increased hepatic β-oxidation of palmitoyl-CoA. This was

accompanied by increased gene expression and/or activity of mitochondrial and peroxisomal fatty acid oxidation enzymes as well as increased levels of Krebs cycle intermediates and ketone bodies (**Paper I**). Consequently, the levels of hepatic triacylglycerols and serum alanine transaminase was normalised, and therefore, increased β-oxidation seems to be the mechanism by which TTA may have a potential in prevention of tamoxifen-induced fatty liver. TTA is also a regulator of mitochondrial biogenesis and is demonstrated to increase mitochondrial respiration, mitochondrial proliferation, mitochondrial DNA content and mitochondrial DNA synthesis enzymes (136, 137). Thus, it is possible that TTA may counteract the influence of tamoxifen on mitochondrial function and thereby prevent tamoxifen-induced fatty liver.

The ability of TTA to prevent tamoxifen-induced hepatic triacylglycerol accumulation makes it a possible candidate for cotreatment with tamoxifen in breast cancer patients, in order to prevent the development of fatty liver. Moreover, this property of TTA also indicates a promising potential in treatment of fatty liver related to the metabolic syndrome.

Hypolipidemic effects of TTA in humans

TTA got approval to be used in human clinical studies from the Norwegian Medicines Agency after no toxic effects were revealed in preclinical toxicology studies in dogs and rats (R.K. Berge, unpublished data). The first clinical safety study with TTA was performed in healthy men receiving TTA (200-1000 mg/day) for 7 days. This study demonstrated that TTA was well tolerated and no adverse side effects were observed (R.K. Berge, unpublished data). Later a study was conducted in HIV-infected patients receiving TTA for 4 weeks together with a cholesterol-lowering diet, resulting in hypolipidemic, antiinflammatory and no adverse side effects of TTA (138).

Also in patients with T2DM, who received a daily 1g dose of TTA for 28 days (**Paper II**), all safety parameters remained within the normal range. In addition, a significant improvement in their serum lipid and lipoprotein profiles was observed, which is in accordance with earlier observations in rats (25). In animals, the serum triacylglycerol level is normally reduced after TTA treatment (25). A reduction in serum triacylglycerol levels was also observed in the dyslipidemic HIV-patients receiving TTA (138), and in another group of patients with dyslipidemia (unpublished data, Rolf K. Berge). However, there was only a tendency of reduced serum triacylglycerol levels in the patients with T2DM after 28 days of TTA treatment (**Paper II**). This tendency was supported by a reduction in serum apo B, which is associated with the triacylglycerol rich VLDL lipoproteins, (**Paper II**). The serum

level of vitamin E has previously been found to correlate with the serum triacylglycerol level (139, 140), and the lowered serum concentration of vitamin E found in the T2DM patients receiving TTA (**Paper II**), therefore adds to the likelihood of a reduced serum triacylglycerol level in these patients. Before TTA treatment, the patients entered a two-week wash out period of lipid lowering drugs. This period may have been too short and could therefore possibly conceal/disturb the potential triacylglycerol lowering effects of TTA.

The National Cholesterol Education Program has identified LDL as the primary target for cholesterol-lowering therapy in order to reduce the risk of CVD (141). Additionally, HDL has been associated with a negative risk of CVD, and hence a reduced LDL/HDL ratio is beneficial. Indeed, total- and LDL-cholesterol was decreased after TTA treatment (**Paper II**), together with an improved (reduced) LDL/HDL ratio. In this study we also observed a decrease in apo B and an increase in apo A1, the main protein components of LDL and HDL respectively, after TTA treatment. Consequently, the apo B/apo A1 ratio was reduced, which is reported to be associated with reduced insulin resistance and risk for the metabolic syndrome and cardiovascular disease (142, 143). Although TTA improves insulin sensitivity in rats (144), we did not observe any effects on glucose metabolism in the type 2 diabetic patients after TTA treatment (**Paper II**). Nevertheless, this study shows TTA as a lipid lowering agent in humans with T2DM. Together with the improved lipid profiles in dyslipidemic HIV patients (138) and another group of dyslipidemic individuals (unpublished data, R.K. Berge), these are uplifting and promising observations.

The role of PPARs in mediating hypolipidemic effects of TTA

The PPARs stimulate the transcription of a great variety of genes in a complex system where tissue specificity and overlapping functions between the PPARs are characteristics. The genes which are under PPAR regulation are involved in many aspects of lipid metabolism, glucose and energy balance, inflammation, differentiation and proliferation (Table 2), reviewed in (59-62). Many of these processes are also affected by TTA (25), and the effects of TTA have indeed been allocated to the function of PPARs, PPARα in special (25, 145). However, with the increasing understanding and focus on the role of PPARδ in fatty acid metabolism and regulation of the metabolic syndrome (62), and since TTA is demonstrated to be a pan-PPAR ligand in various cell lines (26, 27, 75) (**Paper III**), attention should also be put on PPARδ while interpreting the results of TTA treatment.

In rats with DMBA-induced breast cancer TTA prevented hepatic tamoxifen-induced triacylglycerol accumulation (**Paper I**), probably through increased mRNA expression of ACO, CPT-1, CPT-2, CD36, HMG-CoA synthase and liver fatty acid binding protein, as well as increased mitochondrial and peroxisomal β-oxidation of fatty acids. Liver fatty acid oxidation is normally coupled to the action of PPARα (Table 2), so is the expression of the above mentioned genes in the liver (61, 130). Thus, it seems like the effects of TTA at least in part is mediated through PPARα in the liver.

After TTA treatment the serum level of fibrinogen, a blood coagulation factor associated with CVD risk, was significantly reduced in patients with T2DM (**Paper II**). The expression of fibrinogen in human liver and HepG2 cells has previously been demonstrated to be repressed by PPARα (146), and similarly, in a clinical study where patients with impaired glucose tolerance were treated with fenofibrate (PPARα agonist), serum fibrinogen levels were reduced (147). Accordingly, this could also be regarded as a PPARα dependent effect of TTA. The reduced lipid levels, improved lipoprotein profiles and increased level of apo A1 and apo A2 after TTA treatment (**Paper II**) are also in agreement with other clinical studies in patients with obesity related disorders treated with the PPARα agonist GW590735 (148) or fenofibrate (147, 149). As recently reported (148), the PPARδ agonist GW501516 also induce the same reduction in serum lipid levels and improvement of lipoprotein profiles in humans. Similarly, an increase in apo A1 and apo A2 have been reported in monkeys treated with GW501516 (82). The in vitro studies accompanying the clinical data (**Paper II**) show that TTA is a pan-PPAR ligand in liver HepG2 cells, and that in human muscle cells the expression of CPT-1 and CD36, which are regulated by PPARδ in this tissue (148, 150), was increased. This may suggest a role of both PPARα and PPARδ in TTA mediated effects.

Studies in mice revealed that PPARα was involved in, but not essential for increased fatty acid β-oxidation by TTA in liver, as TTA also induced β-oxidation in PPARα deficient mice (**Paper III**). Moreover, the hepatic PPARα expression is lower in humans compared to rodents (151), and since a dominant-negative splice variant of PPARα is present in human liver (151, 152) a more modest role of PPARα in human liver has been suggested (61). This indicate that the role of PPARα in lowering hepatic lipid levels after TTA treatment may be less important, pointing in favour of other mechanisms, possibly involving PPARδ activation. In light of the above mentioned results (**Papers II and III**) (82, 146-150), the complexity of the PPAR system (Table 2), and the fact PPARδ has emerged as a major regulator of lipid

metabolism (62, 125, 153), it seems reasonable to believe that the activity of both PPARα and PPARδ, may be conductive to TTA's mechanism of action.

Some genes involved in lipid metabolism, such as ACO and CPT-1, are regulated by both PPARα (130, 154, 155) and PPARδ (62, 125, 148), but in different tissues. It has been demonstrated that PPARδ may compensate for the loss of PPARα in regulation of fatty acid homeostasis in skeletal muscle of PPARα knock out mice (156). It is possible that this also occurred in the liver of PPARα knock out mice treated with TTA (**Paper III**). The main function of PPARδ seems to be in skeletal muscle and adipose tissue, where increased fatty acid oxidation and improved mitochondrial function have been reported (62). The role of PPARδ in liver appears to be increased fatty acid synthesis at the expense of glucose levels (65), but decreased triacylglycerol accumulation in mouse liver is also observed after PPARδ activation (127). However, one study indicates that the expression of genes that are considered as PPARα targets in the liver is unaffected by PPARδ deficiency (157). In addition, contrary to the compensatory role of PPARδ in skeletal muscle of PPARα knock out mice (156), there are indications for a distinct role of PPARα and PPARδ in skeletal muscle, asserting that PPARδ may not cooperate or compensate for PPARα in this tissue (158).

It has been demonstrated that pharmacological activation of PPARδ or PPARγ improves insulin sensitivity through different metabolic pathways (65). This suggests that PPARδ may also in other situations induce the same net effects as PPARα or PPARγ, although the mechanisms are not the same. Thus, in order to indicate with more certainty that PPARδ compensates for the loss of PPARα in liver, further studies are necessary to determine the specific role of PPARδ in liver, both in wild type and in PPARα deficient animals. PPARδ activation has in fact been demonstrated to induce the expression of PGC-1 (127). In this regard, focus should also be put on mitochondrial function.

Coactivator dependent PPAR activity and the influence of TTA

In order to approach a better mechanistic understanding of TTA's actions, we studied the potential of TTA to stimulate PPAR coactivation by coactivators that are known to play a role in mitochondrial function and energy homeostasis; SRC-1, SRC-2 (114) and PGC-1 (116, 117) in two different cell lines; HepG2 and MCF-7 (**Paper III**). Cell type specific responses were observed, which is not unexpected considering the tissue specific roles of PPARs and coactivators in vivo (Tables 2 and 4). Similar for both cell lines was the potent coactivation

by PGC-1 and poor coactivation by SRC-1, while the combination of these two resulted in synergistic increase in the coactivation, except from PPARδ activation in MCF-7 cells (**Paper III**). Others have reported that PGC-1 is dependent on the interaction with SRC-1 for stimulation of PPARγ activity (114, 123), which is in contrast to our findings of the effects of PGC-1 alone on PPARγ, and which cannot be applied to our observations of PPARα or PPARδ either (**Paper III**). The discrepancy of these results is probably due to the complex PPAR system which is highly dependent on cell types and tissues (59, 61, 62), and the action of coactivators that may be equally complex (114, 116-120).

Addition of specific PPAR agonists or TTA led to minor stimulation of PPARα activity in presence of coactivators in both cell lines (**Paper III**). On the other hand, ligand responsive effects were observed for PPARγ and especially PPARδ. Worth noticing are the increased potency of the ligands in the presence of SRC-2 in MCF-7 cells, and the high level of PPARδ activation (200 fold) obtained by the addition of TTA or PPARδ specific agonist in PGC-1 overexpressing cells (**Paper III**). These in vitro results support that the activity of PPARδ, with a substantial contribution of NR coactivators, PGC-1 in special, might be an important part of TTA's hypolipidemic mechanisms. With regards to the previous discussion of the role of PPARδ in liver lipid metabolism, it is possible that the PPARα independent effects of TTA in liver (**Paper III**) include PGC-1 dependent improvement on mitochondrial function and stimulation of mitochondrial biogenesis, thus contributing to increased mitochondrial β-oxidation and lipid lowering.

The antioxidant, antiinflammatory and hypolipidemic potential of TSA

In an attempt to find a stronger antioxidant than TTA, the modified fatty acid TSA was developed by inserting a selenium atom in the 3-position of the carbon backbone, as selenium is a stronger reducing agent than sulphur. TSA is far less studied that TTA, but as hypothesised, the stronger antioxidant properties of TSA was confirmed in studies performed in vitro (28). In the present study (**Paper IV**) we confirmed the antioxidant effects of TSA and further explored if TSA could hold antiinflammatory and hypolipidemic properties. In activated immune cells isolated from humans, TSA repressed the release of the proinflammatory interleukin (IL)-2 and increased the release of the antiinflammatory IL-10 (**Paper IV**). The release of IL-4, which is traditionally considered as an antiinflammatory cytokine, was also decreased (**Paper IV**). However, studies have indicated both proinflammatory and proatherogenic roles of IL-4 (159-162). Accordingly, the net effect of

TSA mediated cytokine release from activated human immune cells seems to be antiinflammatory, with a potential to be antiatherogenic as well. As pro-oxidants may play a role in the pathogenesis of inflammation (163), it is possible that the antiinflammatory effects of TSA is a consequence of its antioxidant properties. However, with respect to the relevance of PPARs in inflammation, and that the observed antiinflammatory properties of TSA in part coincide with the effects of PPAR activation (164-166), it was interesting to observe that TSA functioned as a pan-PPAR ligand in HepG2 cells (**Paper IV**). Moreover, the antioxidant actions of TSA, including reduced LDL oxidation and increased expression of superoxide dismutases, are also previously demonstrated to occur after PPARα and PPARγ activation (147, 167).

In addition to the antioxidant and antiinflammatory properties of TSA, we found that TSA was able to increase hepatic mitochondrial fatty acid β-oxidation and decrease the level of plasma lipids in rats (**Paper IV**), which are also known effects of TTA (25). The rats were treated with fairly low TSA doses (8-25 $mgKg^{-1}day^{-1}$), compared to the usual TTA dose (150-300 $mgKg^{-1}day^{-1}$) in animal studies, indicating that TSA might be a more potent hypolipidemic agent than TTA. The TSA mediated lipid lowering may be explained by increased expression of the PPAR target genes, ACO, CPT-1 and CPT-2 in vivo, supported by increased expression of the PPAR target genes ACO and CD36 in vitro (**Paper IV**). Thus, it seems like both TTA and TSA may function through PPAR dependent pathways.

Percutaneous coronary intervention is a therapeutic procedure to treat arteries that are narrowed due to the build-up of atherosclerotic plaque. Restenosis may follow after this procedure, and includes some of the same characteristics as atherosclerosis, involving inflammation, oxidative stress and increased proliferation of smooth muscle cells in the arterial wall (14, 29). In the arteries of pigs after percutaneous coronary intervention, TSA prevented smooth muscle cell proliferation and arterial intimal thickening (**Paper IV**), this is also an effect observed after PPARα activation (168). This observation, together with the potential of TSA in counteracting oxidative events, inflammation and hyperlipidemia, which are classified as atherosclerotic risk factors, point towards TSA as an interesting agent for further exploration of anti-atherosclerotic potential. The role of PPARs in this context should also be considered.

Some methodological considerations

In vitro fatty acid administration

The cellular fatty acid uptake of fatty acids administered bound to bovine serum albumin (BSA) and unbound fatty acids has previously been examined (169). It was demonstrated that at concentrations of 200 µM and 500 µM the cellular uptake, and hence the toxicity, of BSA bound fatty acids were significantly lower than unbound fatty acids (169). In some assays of **Paper IV**, TSA was administered at such high doses (100 µM and 500 µM), but in complex with BSA. The BSA complex and the measurement of cell viability after treatment enabled us to rule out toxic effects of TSA in these assays. In other parts of the present investigation (**Papers II, III and IV**) TTA or TSA were administered at lower doses dissolved in either ethanol or dimethylsulfoxide, and toxicity of the solvent in the cell media was prevented by keeping the solvent concentration low (0.1 % v/v).

Transient transfection studies and real time RT-PCR

An important part of the papers in this thesis has been to evaluate the effect of TTA and TSA on PPAR dependent transcriptional activity and the mRNA expression of known PPAR target genes. The approach to these tasks was to perform luciferase reporter driven transient transfection assays, and quantitative real-time RT-PCR.

Transient transfection studies with a firefly luciferase reporter (**Papers II, III and IV**), evaluating PPAR dependent transcriptional activity, was carried out by the SuperFect (Qiagen) procedure, optimised for each cell line. The method allowed for simple analysis of PPAR dependent transcriptional activity in multi-well format with the capacity of analysing several parameters at the same time. The advantage of firefly luciferase as a reporter is the high efficiency and sensitivity (170).

A limitation of this method could be the influence of exogenous ligands on endogenous cellular processes that may influence endogenous ligands and coactivators/corepressors, and subsequently affect the PPAR dependent transcriptional response. Thus, although these data provide valuable indications of PPAR activity, the results must be interpreted keeping in mind that the observed PPAR response probably results from the combined action of the direct binding of the exogenous ligands, and the indirect effects of endogenous ligands and coactivators/corepressors (84).

By accompanying the transfection assays with RT-PCR analysis of the expression of PPAR target genes, the results can be interpreted with more certainty. In most of the present studies, quantitative real time RT-PCR was used for evaluation of mRNA expression.

SYBRgreen (**Paper II**) and TaqMan probes (**Papers I, III, and IV**) were used as the detectable quantifying units, allowing for high sensitivity and specificity (171). Primers and probes were normally designed so that the amplicons cross over exon-exon junctions, favouring cDNA and not genomic DNA amplification (171).

CONCLUSIVE REMARKS

The present thesis has presented two modified fatty acids, TTA and TSA, which are demonstrated to have a possible therapeutic potential in obesity related disorders and risk factors of the metabolic syndrome and cardiovascular disease. Their mechanism of action seems to include the action of PPARs, with contribution of NR coactivators.

Based on the results presented in this thesis, the following can be concluded:

- Increased hepatic triacylglycerol accumulation after tamoxifen is probably a result of increased triacylglycerol biosynthesis combined with unchanged β-oxidation. TTA prevented this development through mechanisms of increased mitochondrial and peroxisomal β-oxidation in the liver.

- TTA has hypolipidemic effects in patients with T2DM, through mechanisms that seem to involve PPAR activation and increased mitochondrial fatty acid oxidation.

- PPARα is involved in, but not essential for the hypolipidemic mechanisms of TTA in rat liver. A dual action of PPARα and PPARδ, with substantial contribution of NR coactivators, and a subsequent increased mitochondrial function appears to be TTA's mechanism of action.

- TSA exert potent antioxidant, antiinflammatory and hypolipidemic properties through mechanisms that are potentially related to increased PPAR activity and mitochondrial fatty acid oxidation.

FUTURE PERSPECTIVES

This thesis provides evidence that TTA and TSA may have a therapeutic potential in obesity related disorders and risk factors of the metabolic syndrome and cardiovascular disease. TTA has been extensively studied in cells, animals (i.a. **Paper I**) and in an exploratory study in patients with T2DM (i.a. **Paper II**), and have provided us with conviction to perform future studies in humans. At present, the individual components of the metabolic syndrome are treated separately; i.e., statins are used for elevated cholesterol, fibrates are used to reduce triacylglycerol, and metformin and thiazolidinediones are used for hyperglycaemia. The wide range of beneficial effects suggested by the response to TTA, calls for larger long-term studies in patients for the treatment of hyperlipidemia, liver fat accumulation, obesity, and insulin resistance.

In order to get a more detailed understanding of TTA's mechanism of action, further studies should also be performed in cells and animals. The specific roles of PPARs should be further investigated, as should PPAR independent mechanisms such as mitochondrial function. In vitro RNA interference techniques for gene silencing could be useful in order to specify the roles of PPAR subtypes in specific cell types. Knock out animals could also be valuable model systems in this respect. Concerning the studies of how TTA affects PPAR activation and coactivation (**Paper III**) by coactivators, some questions remain unanswered. It is still unknown how the interactions between PPARs and coactivators or between two coactivators are influenced by TTA. Amongst other methods Chromatin Immunoprecipitation (ChIP) assays will be appropriate for this approach. As cell type specific effects have been observed with TTA in PPAR activation assays, future studies should also consider this.

TSA was developed as a spin-off of TTA in order to see if the chemical properties of the modifying atom could be reflected in the biochemical properties of the fatty acid. TSA has been less studied than TTA, but promising results in regards to oxidative stress, inflammation and hyperlipidemia have been observed (**Paper IV**), and suggests that TSA deserves future attention. Further studies in cells and animals are needed before TSA are clarified for human studies. As with TTA, it will also be interesting to get further insight in TSA's mechanism of action, involving both PPAR-dependent and PPAR-independent mechanisms.

The occurrence of obesity related disorders and risk factors of the metabolic syndrome and cardiovascular disease are steadily increasing all over the world. In this regard, it will be interesting to follow TTA and TSA in future studies with the overall aim to find therapeutic alternatives.

ERRATA

Thesis

Page 34, results, line 7:

“, but” should be replaced by “.” and “only” should be removed.

Page 34, results, line 8:

After “PPARδ” the following should be inserted: “and PPARγ. In MCF-7 cells the effect was most pronounced on PPARδ”.

Paper I

Page 2223, abstract, line 13:

“from” should be replaced by “to”.

Page 2224, first column, line 25:

“nitric oxide” should be replaced by “nitrous oxide”

Page 2224, second column, lines 10-13:

“; And glyceraldehyde-3-phosphate dehydrogenase forward (59-TGC ACC ACC AAC TGC TTA GC-39) reverse (59-CAG TCT TCT GAG TGG CAG TGA TG-39) and probe (59-TGG AAG GGC TCA TGA CCA CAG TCC A-39)” should be removed.

Page 2224, second column, line 30:

“(p/n: 4308313, Applied Biosystems)” should be inserted after “glyceraldehyde-3-phosphate dehydrogenase”.

Paper II

Page 6, line 3:

“PAPRδ” should be replaced by “PPARδ”.

Paper III

Page 3, line 13:

The first "PPARγ" should be replaced by "PPARδ" so that the list of PPARs says "PPARα, PPARδ or PPARγ".

Page 9, line 2 from the bottom:

"and PPARγ" should be inserted after "PPARδ", and "PPARδ activity" should be inserted after "reaching".

Page 10, discussion, lines 4-5:

"and PPARγ" should be moved to after "PPARδ".

Page 12, line 5 from the bottom:

"and PPARγ" should be inserted after "PPARδ".

Paper IV

Supplemental materials and methods, page 8, line 4:

"and serum transaminases" should be removed.

Supplemental materials and methods, page 9, line 15:

"NO" should be replaced by "N_2O".

REFERENCES

1. **James PT, Rigby N, Leach R** 2004 The obesity epidemic, metabolic syndrome and future prevention strategies. Eur J Cardiovasc Prev Rehabil 11:3-8
2. **Haslam DW, James WP** 2005 Obesity. Lancet 366:1197-209
3. **WHO** 1999 World Health Organisation. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: Diagnosis and classification of diabetes mellitus. WHO bull:1-59
4. **Wellen KE, Hotamisligil GS** 2005 Inflammation, stress, and diabetes. J Clin Invest 115:1111-9
5. **Hotamisligil GS, Shargill NS, Spiegelman BM** 1993 Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259:87-91
6. **Trayhurn P, Wood IS** 2005 Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem. Soc. Trans. 33:1078-1081
7. **Boden G, Chen X** 1995 Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. J Clin Invest 96:1261-8
8. **Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI** 1999 Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. J Clin Invest 103:253-9
9. **Boden G, Cheung P, Stein TP, Kresge K, Mozzoli M** 2002 FFA cause hepatic insulin resistance by inhibiting insulin suppression of glycogenolysis. Am J Physiol Endocrinol Metab 283:E12-9
10. **Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M** 1998 Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 339:229-34
11. **Miettinen H, Lehto S, Salomaa V, Mahonen M, Niemela M, Haffner SM, Pyorala K, Tuomilehto J** 1998 Impact of diabetes on mortality after the first myocardial infarction. The FINMONICA Myocardial Infarction Register Study Group. Diabetes Care 21:69-75
12. **Betteridge J** 2005 Benefits of lipid-lowering therapy in patients with type 2 diabetes mellitus. Am J Med 118 Suppl 12A:10-5
13. **Stocker R, Keaney JF, Jr.** 2004 Role of Oxidative Modifications in Atherosclerosis. Physiol. Rev. 84:1381-1478
14. **Hansson GK** 2005 Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352:1685-95
15. **Marchesini G, Babini M** 2006 Nonalcoholic fatty liver disease and the metabolic syndrome. Minerva Cardioangiol 54:229-39
16. **Wanless IR, Lentz JS** 1990 Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. Hepatology 12:1106-10
17. **James O, Day C** 1999 Non-alcoholic steatohepatitis: another disease of affluence. Lancet 353:1634-6
18. **Angulo P, Lindor KD** 2002 Non-alcoholic fatty liver disease. J Gastroenterol Hepatol 17 Suppl:S186-90
19. **Angulo P** 2002 Nonalcoholic fatty liver disease. N Engl J Med 346:1221-31
20. **Nguyen MC, Stewart RB, Banerji MA, Gordon DH, Kral JG** 2001 Relationships between tamoxifen use, liver fat and body fat distribution in women with breast cancer. Int J Obes Relat Metab Disord 25:296-8

21. **Ogawa Y, Murata Y, Nishioka A, Inomata T, Yoshida S** 1998 Tamoxifen-induced fatty liver in patients with breast cancer. Lancet 351:725
22. **Berge RK, Aarsland A, Kryvi H, Bremer J, Aarsaether N** 1989 Alkylthioacetic acid (3-thia fatty acids)--a new group of non-beta-oxidizable, peroxisome-inducing fatty acid analogues. I. A study on the structural requirements for proliferation of peroxisomes and mitochondria in rat liver. Biochim Biophys Acta 1004:345-56
23. **Madsen L, Froyland L, Grav HJ, Berge RK** 1997 Up-regulated delta 9-desaturase gene expression by hypolipidemic peroxisome-proliferating fatty acids results in increased oleic acid content in liver and VLDL: accumulation of a delta 9-desaturated metabolite of tetradecylthioacetic acid. J Lipid Res 38:554-63
24. **Gudbrandsen OA, Dyroy E, Bohov P, Skorve J, Berge RK** 2005 The metabolic effects of thia fatty acids in rat liver depend on the position of the sulfur atom. Chem Biol Interact 155:71-81
25. **Berge RK, Skorve J, Tronstad KJ, Berge K, Gudbrandsen OA, Grav H** 2002 Metabolic effects of thia fatty acids. Curr Opin Lipidol 13:295-304.
26. **Westergaard M, Henningsen J, Svendsen ML, Johansen C, Jensen UB, Schroder HD, Kratchmarova I, Berge RK, Iversen L, Bolund L, Kragballe K, Kristiansen K** 2001 Modulation of keratinocyte gene expression and differentiation by PPAR-selective ligands and tetradecylthioacetic acid. J Invest Dermatol 116:702-12.
27. **Berge K, Tronstad KJ, Flindt EN, Rasmussen TH, Madsen L, Kristiansen K, Berge RK** 2001 Tetradecylthioacetic acid inhibits growth of rat glioma cells ex vivo and in vivo via PPAR-dependent and PPAR-independent pathways. Carcinogenesis 22:1747-55.
28. **Muna ZA, Bolann BJ, Chen X, Songstad J, Berge RK** 2000 Tetradecylthioacetic acid and tetradecylselenoacetic acid inhibit lipid peroxidation and interact with superoxide radical. Free Radic Biol Med 28:1068-78
29. **Pettersen RJ, Muna ZA, Kuiper KK, Svendsen E, Muller F, Aukrust P, Berge RK, Nordrehaug JE** 2001 Sustained retention of tetradecylthioacetic acid after local delivery reduces angioplasty-induced coronary stenosis in the minipig. Cardiovasc Res 52:306-13.
30. **Bonen A, Chabowski A, Luiken JJ, Glatz JF** 2007 Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. Physiology (Bethesda) 22:15-29
31. **Stremmel W, Strohmeyer G, Borchard F, Kochwa S, Berk PD** 1985 Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. Proc Natl Acad Sci U S A 82:4-8
32. **Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA** 1993 Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J Biol Chem 268:17665-8
33. **Harmon CM, Abumrad NA** 1993 Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. J Membr Biol 133:43-9
34. **Harmon CM, Luce P, Beth AH, Abumrad NA** 1991 Labeling of adipocyte membranes by sulfo-N-succinimidyl derivatives of long-chain fatty acids: inhibition of fatty acid transport. J Membr Biol 121:261-8
35. **Schaffer JE, Lodish HF** 1994 Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. Cell 79:427-36

36. **Gimeno RE, Ortegon AM, Patel S, Punreddy S, Ge P, Sun Y, Lodish HF, Stahl A** 2003 Characterization of a heart-specific fatty acid transport protein. J Biol Chem 278:16039-44
37. **Hirsch D, Stahl A, Lodish HF** 1998 A family of fatty acid transporters conserved from mycobacterium to man. Proc Natl Acad Sci U S A 95:8625-9
38. **Ockner RK, Manning JA, Poppenhausen RB, Ho WK** 1972 A binding protein for fatty acids in cytosol of intestinal mucosa, liver, myocardium, and other tissues. Science 177:56-8
39. **Weisiger RA** 2007 Mechanisms of intracellular fatty acid transport: role of cytoplasmic-binding proteins. J Mol Neurosci 33:42-4
40. **Groot PH, Scholte HR, Hulsmann WC** 1976 Fatty acid activation: specificity, localization, and function. Adv Lipid Res 14:75-126
41. **Eaton S** 2002 Control of mitochondrial beta-oxidation flux. Prog Lipid Res 41:197-239.
42. **Aas M** 1971 Organ and subcellular distribution of fatty acid activating enzymes in the rat. Biochim Biophys Acta 231:32-47
43. **Lazarow PB** 1978 Rat liver peroxisomes catalyze the beta oxidation of fatty acids. J Biol Chem 253:1522-8
44. **Wanders RJ, Vreken P, Ferdinandusse S, Jansen GA, Waterham HR, van Roermund CW, Van Grunsven EG** 2001 Peroxisomal fatty acid alpha- and beta-oxidation in humans: enzymology, peroxisomal metabolite transporters and peroxisomal diseases. Biochem Soc Trans 29:250-67
45. **Wurtz JM, Bourguet W, Renaud JP, Vivat V, Chambon P, Moras D, Gronemeyer H** 1996 A canonical structure for the ligand-binding domain of nuclear receptors. Nat Struct Biol 3:87-94.
46. **Moras D, Gronemeyer H** 1998 The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol 10:384-91.
47. **Laudet V** 1997 Evolution of the nuclear receptor superfamily: early diversification from an ancestral orphan receptor. J Mol Endocrinol 19:207-26.
48. **Committee NRN, Laudet V, Auwerx J, Gustafsson JA, Wahli W** 1999 A Unified Nomenclature System for the Nuclear Receptor Superfamily. Cell 97:161
49. **Aranda A, Pascual A** 2001 Nuclear hormone receptors and gene expression. Physiol Rev 81:1269-304.
50. **Issemann I, Green S** 1990 Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645-50
51. **Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W** 1992 Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 68:879-87
52. **Sher T, Yi HF, McBride OW, Gonzalez FJ** 1993 cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor. Biochemistry 32:5598-604.
53. **Schmidt A, Endo N, Rutledge SJ, Vogel R, Shinar D, Rodan GA** 1992 Identification of a new member of the steroid hormone receptor superfamily that is activated by a peroxisome proliferator and fatty acids. Mol Endocrinol 6:1634-41
54. **Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K, Evans RM** 1994 Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc Natl Acad Sci U S A 91:7355-9
55. **Greene ME, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K, Hsieh L, Greene G, Nimer SD** 1995 Isolation of the human peroxisome proliferator activated

receptor gamma cDNA: expression in hematopoietic cells and chromosomal mapping. Gene Expr 4:281-99

56. **Fajas L, Fruchart JC, Auwerx J** 1998 PPARgamma3 mRNA: a distinct PPARgamma mRNA subtype transcribed from an independent promoter. FEBS Lett 438:55-60
57. **Elbrecht A, Chen Y, Cullinan CA, Hayes N, Leibowitz M, Moller DE, Berger J** 1996 Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma 1 and gamma 2. Biochem Biophys Res Commun 224:431-7
58. **Mukherjee R, Jow L, Croston GE, Paterniti JR, Jr.** 1997 Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARgamma2 versus PPARgamma1 and activation with retinoid X receptor agonists and antagonists. J Biol Chem 272:8071-6
59. **Semple RK, Chatterjee VK, O'Rahilly S** 2006 PPAR gamma and human metabolic disease. J Clin Invest 116:581-9
60. **Mandard S, Muller M, Kersten S** 2004 Peroxisome proliferator-activated receptor alpha target genes. Cell Mol Life Sci 61:393-416
61. **Lefebvre P, Chinetti G, Fruchart JC, Staels B** 2006 Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. J Clin Invest 116:571-80
62. **Barish GD, Narkar VA, Evans RM** 2006 PPAR delta: a dagger in the heart of the metabolic syndrome. J Clin Invest 116:590-7
63. **Juge-Aubry C, Pernin A, Favez T, Burger AG, Wahli W, Meier CA, Desvergne B** 1997 DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5'-flanking region. Journal of Biological Chemistry 272:25252-9
64. **Libby P, Plutzky J** 2007 Inflammation in diabetes mellitus: role of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma agonists. Am J Cardiol 99:27B-40B
65. **Lee CH, Olson P, Hevener A, Mehl I, Chong LW, Olefsky JM, Gonzalez FJ, Ham J, Kang H, Peters JM, Evans RM** 2006 PPARdelta regulates glucose metabolism and insulin sensitivity. Proc Natl Acad Sci U S A 103:3444-9
66. **Lee CH, Chawla A, Urbiztondo N, Liao D, Boisvert WA, Evans RM** 2003 Transcriptional repression of atherogenic inflammation: modulation by PPARdelta. Science 302:453-7
67. **Issemann I, Prince RA, Tugwood JD, Green S** 1993 The peroxisome proliferator-activated receptor:retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. J Mol Endocrinol 11:37-47
68. **Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA** 1995 An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270:12953-6
69. **Berger J, Bailey P, Biswas C, Cullinan CA, Doebber TW, Hayes NS, Saperstein R, Smith RG, Leibowitz MD** 1996 Thiazolidinediones produce a conformational change in peroxisomal proliferator-activated receptor-gamma: binding and activation correlate with antidiabetic actions in db/db mice. Endocrinology 137:4189-95
70. **Berger J, Leibowitz MD, Doebber TW, Elbrecht A, Zhang B, Zhou G, Biswas C, Cullinan CA, Hayes NS, Li Y, Tanen M, Ventre J, Wu MS, Berger GD, Mosley R, Marquis R, Santini C, Sahoo SP, Tolman RL, Smith RG, Moller DE** 1999 Novel peroxisome proliferator-activated receptor (PPAR) gamma and PPARdelta ligands produce distinct biological effects. J Biol Chem 274:6718-25.

71. **Leibowitz MD, Fievet C, Hennuyer N, Peinado-Onsurbe J, Duez H, Bergera J, Cullinan CA, Sparrow CP, Baffic J, Berger GD, Santini C, Marquis RW, Tolman RL, Smith RG, Moller DE, Auwerx J** 2000 Activation of PPARdelta alters lipid metabolism in db/db mice. FEBS Lett 473:333-6
72. **Kliewer SA, Umesono K, Noonan DJ, Heyman RA, Evans RM** 1992 Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 358:771-4.
73. **Burns KA, Vanden Heuvel JP** 2007 Modulation of PPAR activity via phosphorylation. Biochim Biophys Acta 1771:952-60
74. **Yu K, Bayona W, Kallen CB, Harding HP, Ravera CP, McMahon G, Brown M, Lazar MA** 1995 Differential activation of peroxisome proliferator-activated receptors by eicosanoids. J Biol Chem 270:23975-83
75. **Forman BM, Chen J, Evans RM** 1997 Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. Proc Natl Acad Sci U S A 94:4312-7
76. **Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM** 1997 Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci U S A 94:4318-23
77. **Krey G, Braissant O, L'Horset F, Kalkhoven E, Perroud M, Parker MG, Wahli W** 1997 Fatty Acids, Eicosanoids, and Hypolipidemic Agents Identified as Ligands of Peroxisome Proliferator-Activated Receptors by Coactivator-Dependent Receptor Ligand Assay. Mol Endocrinol 11:779-791
78. **Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM** 1998 Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. Cell 93:229-40
79. **Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM** 1995 A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell 83:813-9
80. **Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM** 1995 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell 83:803-12
81. **Brown PJ, Winegar DA, Plunket KD, Moore LB, Lewis MC, Wilson JG, Sundseth SS, Koble CS, Wu Z, Chapman JM, Lehmann JM, Kliewer SA, Willson TM** 1999 A ureido-thioisobutyric acid (GW9578) is a subtype-selective PPARalpha agonist with potent lipid-lowering activity. J Med Chem 42:3785-8
82. **Oliver WR, Jr., Shenk JL, Snaith MR, Russell CS, Plunket KD, Bodkin NL, Lewis MC, Winegar DA, Sznaidman ML, Lambert MH, Xu HE, Sternbach DD, Kliewer SA, Hansen BC, Willson TM** 2001 A selective peroxisome proliferator-activated receptor delta agonist promotes reverse cholesterol transport. Proc Natl Acad Sci U S A 98:5306-11
83. **Lambe KG, Tugwood JD** 1996 A human peroxisome-proliferator-activated receptor-gamma is activated by inducers of adipogenesis, including thiazolidinedione drugs. Eur J Biochem 239:1-7
84. **Lemberger T, Desvergne B, Wahli W** 1996 Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol 12:335-63
85. **IJpenberg A, Jeannin E, Wahli W, Desvergne B** 1997 Polarity and specific sequence requirements of peroxisome proliferator- activated receptor (PPAR)/retinoid

X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. J Biol Chem 272:20108-17.

86. **Palmer CN, Hsu MH, Griffin HJ, Johnson EF** 1995 Novel sequence determinants in peroxisome proliferator signaling. J Biol Chem 270:16114-21
87. **Heery DM, Kalkhoven E, Hoare S, Parker MG** 1997 A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature 387:733-6
88. **Nolte RT, Wisely GB, Westin S, Cobb JE, Lambert MH, Kurokawa R, Rosenfeld MG, Willson TM, Glass CK, Milburn MV** 1998 Ligand binding and co-activator assembly of the peroxisome proliferator- activated receptor-gamma. Nature 395:137-43.
89. **Bramlett KS, Burris TP** 2002 Effects of selective estrogen receptor modulators (SERMs) on coactivator nuclear receptor (NR) box binding to estrogen receptors. Mol Genet Metab 76:225-33
90. **Chang C, Norris JD, Gron H, Paige LA, Hamilton PT, Kenan DJ, Fowlkes D, McDonnell DP** 1999 Dissection of the LXXLL nuclear receptor-coactivator interaction motif using combinatorial peptide libraries: discovery of peptide antagonists of estrogen receptors alpha and beta. Mol Cell Biol 19:8226-39
91. **Lonard DM, O'Malley BW** 2005 Expanding functional diversity of the coactivators. Trends Biochem Sci 30:126-32
92. **Cronet P, Petersen JF, Folmer R, Blomberg N, Sjoblom K, Karlsson U, Lindstedt EL, Bamberg K** 2001 Structure of the PPARalpha and -gamma ligand binding domain in complex with AZ 242; ligand selectivity and agonist activation in the PPAR family. Structure 9:699-706
93. **Xu HE, Lambert MH, Montana VG, Parks DJ, Blanchard SG, Brown PJ, Sternbach DD, Lehmann JM, Wisely GB, Willson TM, Kliewer SA, Milburn MV** 1999 Molecular recognition of fatty acids by peroxisome proliferator-activated receptors. Mol Cell 3:397-403
94. **Yu S, Reddy JK** 2007 Transcription coactivators for peroxisome proliferator-activated receptors. Biochim Biophys Acta 1771:936-51
95. **Kodera Y, Takeyama K, Murayama A, Suzawa M, Masuhiro Y, Kato S** 2000 Ligand type-specific interactions of peroxisome proliferator-activated receptor gamma with transcriptional coactivators. J Biol Chem 275:33201-4.
96. **Camp HS, Li O, Wise SC, Hong YH, Frankowski CL, Shen X, Vanbogelen R, Leff T** 2000 Differential activation of peroxisome proliferator-activated receptor-gamma by troglitazone and rosiglitazone. Diabetes 49:539-47.
97. **Dowell P, Peterson VJ, Zabriskie TM, Leid M** 1997 Ligand-induced peroxisome proliferator-activated receptor alpha conformational change. J Biol Chem 272:2013-20.
98. **Onate SA, Tsai SY, Tsai MJ, O'Malley BW** 1995 Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 270:1354-7
99. **Hong H, Kohli K, Trivedi A, Johnson DL, Stallcup MR** 1996 GRIP1, a novel mouse protein that serves as a transcriptional coactivator in yeast for the hormone binding domains of steroid receptors. Proc Natl Acad Sci U S A 93:4948-52
100. **Voegel JJ, Heine MJ, Zechel C, Chambon P, Gronemeyer H** 1996 TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. Embo J 15:3667-75
101. **Guan XY, Xu J, Anzick SL, Zhang H, Trent JM, Meltzer PS** 1996 Hybrid selection of transcribed sequences from microdissected DNA: isolation of genes within amplified region at 20q11-q13.2 in breast cancer. Cancer Res 56:3446-50
102. **Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y, Evans RM** 1997 Nuclear receptor coactivator ACTR is a novel histone

acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. Cell 90:569-80

103. **Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS** 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277:965-8
104. **Zhu Y, Qi C, Calandra C, Rao MS, Reddy JK** 1996 Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferator-activated receptor gamma. Gene Expr 6:185-95
105. **Xu J, Li Q** 2003 Review of the in vivo functions of the p160 steroid receptor coactivator family. Mol Endocrinol 17:1681-92
106. **Chen D, Ma H, Hong H, Koh SS, Huang SM, Schurter BT, Aswad DW, Stallcup MR** 1999 Regulation of transcription by a protein methyltransferase. Science 284:2174-7
107. **Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK, Rosenfeld MG** 1996 A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85:403-14
108. **Spencer TE, Jenster G, Burcin MM, Allis CD, Zhou J, Mizzen CA, McKenna NJ, Onate SA, Tsai SY, Tsai MJ, O'Malley BW** 1997 Steroid receptor coactivator-1 is a histone acetyltransferase. Nature 389:194-8
109. **Dowell P, Ishmael JE, Avram D, Peterson VJ, Nevrivy DJ, Leid M** 1997 p300 functions as a coactivator for the peroxisome proliferator-activated receptor alpha. J Biol Chem 272:33435-43
110. **Lim HJ, Moon I, Han K** 2004 Transcriptional cofactors exhibit differential preference toward peroxisome proliferator-activated receptors alpha and delta in uterine cells. Endocrinology 145:2886-95
111. **Yang W, Rachez C, Freedman LP** 2000 Discrete roles for peroxisome proliferator-activated receptor gamma and retinoid X receptor in recruiting nuclear receptor coactivators. Mol Cell Biol 20:8008-17
112. **Leers J, Treuter E, Gustafsson JA** 1998 Mechanistic principles in NR box-dependent interaction between nuclear hormone receptors and the coactivator TIF2. Mol Cell Biol 18:6001-13
113. **Louet JF, Coste A, Amazit L, Tannour-Louet M, Wu RC, Tsai SY, Tsai MJ, Auwerx J, O'Malley BW** 2006 Oncogenic steroid receptor coactivator-3 is a key regulator of the white adipogenic program. Proc Natl Acad Sci U S A 103:17868-73
114. **Picard F, Gehin M, Annicotte J, Rocchi S, Champy MF, O'Malley BW, Chambon P, Auwerx J** 2002 SRC-1 and TIF2 control energy balance between white and brown adipose tissues. Cell 111:931-41.
115. **Wang Z, Qi C, Krones A, Woodring P, Zhu X, Reddy JK, Evans RM, Rosenfeld MG, Hunter T** 2006 Critical roles of the p160 transcriptional coactivators p/CIP and SRC-1 in energy balance. Cell Metab 3:111-22
116. **Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM** 1998 A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 92:829-39
117. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM** 1999 Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 98:115-24
118. **Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, Oh KS, Koh EH, Won JC, Kim MS, Oh GT, Yoon M, Lee KU, Park JY** 2006 AMPK activation increases fatty acid

oxidation in skeletal muscle by activating PPARalpha and PGC-1. Biochem Biophys Res Commun 340:291-5

119. **Koo SH, Satoh H, Herzig S, Lee CH, Hedrick S, Kulkarni R, Evans RM, Olefsky J, Montminy M** 2004 PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. Nat Med 10:530-4
120. **Yoon JC, Puigserver P, Chen G, Donovan J, Wu Z, Rhee J, Adelmant G, Stafford J, Kahn CR, Granner DK, Newgard CB, Spiegelman BM** 2001 Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. Nature 413:131-8
121. **Lin J, Puigserver P, Donovan J, Tarr P, Spiegelman BM** 2002 Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. J Biol Chem 277:1645-8
122. **Andersson U, Scarpulla RC** 2001 Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. Mol Cell Biol 21:3738-49
123. **Puigserver P, Adelmant G, Wu Z, Fan M, Xu J, O'Malley B, Spiegelman BM** 1999 Activation of PPARgamma coactivator-1 through transcription factor docking. Science 286:1368-71
124. **Monsalve M, Wu Z, Adelmant G, Puigserver P, Fan M, Spiegelman BM** 2000 Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. Mol Cell 6:307-16
125. **Wang YX, Lee CH, Tiep S, Yu RT, Ham J, Kang H, Evans RM** 2003 Peroxisome-proliferator-activated receptor delta activates fat metabolism to prevent obesity. Cell 113:159-70
126. **Vega RB, Huss JM, Kelly DP** 2000 The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 20:1868-76
127. **Tanaka T, Yamamoto J, Iwasaki S, Asaba H, Hamura H, Ikeda Y, Watanabe M, Magoori K, Ioka RX, Tachibana K, Watanabe Y, Uchiyama Y, Sumi K, Iguchi H, Ito S, Doi T, Hamakubo T, Naito M, Auwerx J, Yanagisawa M, Kodama T, Sakai J** 2003 Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. Proc Natl Acad Sci U S A 100:15924-9
128. **Aas V, Kase ET, Solberg R, Jensen J, Rustan AC** 2004 Chronic hyperglycaemia promotes lipogenesis and triacylglycerol accumulation in human skeletal muscle cells. Diabetologia 47:1452-61
129. 1998 Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 351:1451-67
130. **Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, Fernandez-Salguero PM, Westphal H, Gonzalez FJ** 1995 Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol Cell Biol 15:3012-22
131. **Larosche I, Letteron P, Fromenty B, Vadrot N, Abbey-Toby A, Feldmann G, Pessayre D, Mansouri A** 2007 Tamoxifen inhibits topoisomerases, depletes mitochondrial DNA, and triggers steatosis in mouse liver. J Pharmacol Exp Ther 321:526-35
132. **Lelliott CJ, Lopez M, Curtis RK, Parker N, Laudes M, Yeo G, Jimenez-Linan M, Grosse J, Saha AK, Wiggins D, Hauton D, Brand MD, O'Rahilly S, Griffin JL, Gibbons GF, Vidal-Puig A** 2005 Transcript and metabolite analysis of the effects of

tamoxifen in rat liver reveals inhibition of fatty acid synthesis in the presence of hepatic steatosis. Faseb J 19:1108-19

133. **Trickett JI, Patel DD, Knight BL, Saggerson ED, Gibbons GF, Pease RJ** 2001 Characterization of the rodent genes for arylacetamide deacetylase, a putative microsomal lipase, and evidence for transcriptional regulation. J Biol Chem 276:39522-32
134. **Fisher EA, Ginsberg HN** 2002 Complexity in the Secretory Pathway: The Assembly and Secretion of Apolipoprotein B-containing Lipoproteins. J. Biol. Chem. 277:17377-17380
135. **Cardoso CM, Custodio JB, Almeida LM, Moreno AJ** 2001 Mechanisms of the deleterious effects of tamoxifen on mitochondrial respiration rate and phosphorylation efficiency. Toxicol Appl Pharmacol 176:145-52
136. **Elholm M, Hollas H, Issalene C, Barroso JF, Berge RK, Flatmark T** 2001 Transient up-regulation of liver mitochondrial thymidine kinase activity in proliferating mitochondria. IUBMB Life 51:99-104
137. **Grav HJ, Tronstad KJ, Gudbrandsen OA, Berge K, Fladmark KE, Martinsen TC, Waldum H, Wergedahl H, Berge RK** 2003 Changed energy state and increased mitochondrial beta-oxidation rate in liver of rats associated with lowered proton electrochemical potential and stimulated uncoupling protein 2 (UCP-2) expression: evidence for peroxisome proliferator-activated receptor-alpha independent induction of UCP-2 expression. J Biol Chem 278:30525-33
138. **Fredriksen J, Ueland T, Dyroy E, Halvorsen B, Melby K, Melbye L, Skalhegg BS, Bohov P, Skorve J, Berge RK, Aukrust P, Froland SS** 2004 Lipid-lowering and anti-inflammatory effects of tetradecylthioacetic acid in HIV-infected patients on highly active antiretroviral therapy. Eur J Clin Invest 34:709-15
139. **Horwitt MK, Harvey CC, Dahm CH, Jr., Searcy MT** 1972 Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann N Y Acad Sci 203:223-36
140. **Muna ZA, Gudbrandsen OA, Wergedahl H, Bohov P, Skorve J, Berge RK** 2002 Inhibition of rat lipoprotein oxidation after tetradecylthioacetic acid feeding. Biochem Pharmacol 63:1127-35
141. **ATPIII** 2002 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation 106:3143-3421
142. **Sniderman AD, Faraj M** 2007 Apolipoprotein B, apolipoprotein A-I, insulin resistance and the metabolic syndrome. Curr Opin Lipidol 18:633-7
143. **Sierra-Johnson J, Romero-Corral A, Somers VK, Lopez-Jimenez F, Walldius G, Hamsten A, Hellenius ML, Fisher RM** 2007 ApoB/apoA-I ratio: an independent predictor of insulin resistance in US non-diabetic subjects. Eur Heart J 28:2637-43
144. **Madsen L, Guerre-Millo M, Flindt EN, Berge K, Tronstad KJ, Bergene E, Sebokova E, Rustan AC, Jensen J, Mandrup S, Kristiansen K, Klimes I, Staels B, Berge RK** 2002 Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance. J Lipid Res 43:742-50.
145. **Raspe E, Madsen L, Lefebvre AM, Leitersdorf I, Gelman L, Peinado-Onsurbe J, Dallongeville J, Fruchart JC, Berge R, Staels B** 1999 Modulation of rat liver apolipoprotein gene expression and serum lipid levels by tetradecylthioacetic acid (TTA) via PPARalpha activation. J Lipid Res 40:2099-110
146. **Gervois P, Vu-Dac N, Kleemann R, Kockx M, Dubois G, Laine B, Kosykh V, Fruchart JC, Kooistra T, Staels B** 2001 Negative regulation of human fibrinogen

gene expression by peroxisome proliferator-activated receptor alpha agonists via inhibition of CCAAT box/enhancer-binding protein beta. J Biol Chem 276:33471-7

147. **Okopien B, Krysiak R, Herman ZS** 2006 Effects of short-term fenofibrate treatment on circulating markers of inflammation and hemostasis in patients with impaired glucose tolerance. J Clin Endocrinol Metab 91:1770-8
148. **Riserus U, Sprecher D, Johnson T, Olson E, Hirschberg S, Liu A, Fang Z, Hegde P, Richards D, Sarov-Blat L, Strum JC, Basu S, Cheeseman J, Fielding BA, Humphreys SM, Danoff T, Moore NR, Murgatroyd P, O'Rahilly S, Sutton P, Willson T, Hassall D, Frayn KN, Karpe F** 2007 Activation of PPAR{delta} promotes reversal of multiple metabolic abnormalities, reduces oxidative stress and increases fatty acid oxidation in moderately obese men. Diabetes
149. **Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, Staels B, Auwerx J** 1995 Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. J Clin Invest 96:741-50
150. **Holst D, Luquet S, Nogueira V, Kristiansen K, Leverve X, Grimaldi PA** 2003 Nutritional regulation and role of peroxisome proliferator-activated receptor delta in fatty acid catabolism in skeletal muscle. Biochim Biophys Acta 1633:43-50
151. **Palmer CNA, Hsu M-H, Griffin KJ, Raucy JL, Johnson EF** 1998 Peroxisome Proliferator Activated Receptor-alpha Expression in Human Liver. Mol Pharmacol 53:14-22
152. **Gervois P, Torra IP, Chinetti G, Grotzinger T, Dubois G, Fruchart J-C, Fruchart-Najib J, Leitersdorf E, Staels B** 1999 A Truncated Human Peroxisome Proliferator-Activated Receptor {alpha} Splice Variant with Dominant Negative Activity. Mol Endocrinol 13:1535-1549
153. **Luquet S, Lopez-Soriano J, Holst D, Gaudel C, Jehl-Pietri C, Fredenrich A, Grimaldi PA** 2004 Roles of peroxisome proliferator-activated receptor delta (PPARdelta) in the control of fatty acid catabolism. A new target for the treatment of metabolic syndrome. Biochimie 86:833-7
154. **Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S** 1992 The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. Embo J 11:433-9
155. **Osumi T, Wen JK, Hashimoto T** 1991 Two cis-acting regulatory sequences in the peroxisome proliferator-responsive enhancer region of rat acyl-CoA oxidase gene. Biochem Biophys Res Commun 175:866-71
156. **Muoio DM, MacLean PS, Lang DB, Li S, Houmard JA, Way JM, Winegar DA, Corton JC, Dohm GL, Kraus WE** 2002 Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. J Biol Chem 277:26089-97
157. **Peters JM, Aoyama T, Burns AM, Gonzalez FJ** 2003 Bezafibrate is a dual ligand for PPARalpha and PPARbeta: studies using null mice. Biochim Biophys Acta 1632:80-9
158. **Bedu E, Desplanches D, Pequignot J, Bordier B, Desvergne B** 2007 Double gene deletion reveals the lack of cooperation between PPARalpha and PPARbeta in skeletal muscle. Biochem Biophys Res Commun 357:877-81
159. **Davenport P, Tipping PG** 2003 The role of interleukin-4 and interleukin-12 in the progression of atherosclerosis in apolipoprotein E-deficient mice. Am J Pathol 163:1117-25

160. **George J, Shoenfeld Y, Gilburd B, Afek A, Shaish A, Harats D** 2000 Requisite role for interleukin-4 in the acceleration of fatty streaks induced by heat shock protein 65 or Mycobacterium tuberculosis. Circ Res 86:1203-10
161. **King VL, Szilvassy SJ, Daugherty A** 2002 Interleukin-4 deficiency decreases atherosclerotic lesion formation in a site-specific manner in female LDL receptor-/- mice. Arterioscler Thromb Vasc Biol 22:456-61
162. **Walch L, Massade L, Dufilho M, Brunet A, Rendu F** 2006 Pro-atherogenic effect of interleukin-4 in endothelial cells: modulation of oxidative stress, nitric oxide and monocyte chemoattractant protein-1 expression. Atherosclerosis 187:285-91
163. **Geronikaki AA, Gavalas AM** 2006 Antioxidants and inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. Comb Chem High Throughput Screen 9:425-42
164. **Moraes LA, Piqueras L, Bishop-Bailey D** 2006 Peroxisome proliferator-activated receptors and inflammation. Pharmacol Ther 110:371-85
165. **Stienstra R, Duval C, Müller M, Kersten S** 2007 PPARs, obesity and inflammation. PPAR Res 2007:95974
166. **Thompson PW, Bayliffe AI, Warren AP, Lamb JR** 2007 Interleukin-10 is upregulated by nanomolar rosiglitazone treatment of mature dendritic cells and human CD4+ T cells. Cytokine 39:184-91
167. **Inoue I, Goto S, Matsunaga T, Nakajima T, Awata T, Hokari S, Komoda T, Katayama S** 2001 The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma increase Cu2+,Zn2+-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. Metabolism 50:3-11
168. **Gizard F, Amant C, Barbier O, Bellosta S, Robillard R, Percevault F, Sevestre H, Krimpenfort P, Corsini A, Rochette J, Glineur C, Fruchart JC, Torpier G, Staels B** 2005 PPAR alpha inhibits vascular smooth muscle cell proliferation underlying intimal hyperplasia by inducing the tumor suppressor p16INK4a. J Clin Invest 115:3228-38
169. **Tronstad KJ, Berge K, Flindt EN, Kristiansen K, Berge RK** 2001 Optimization of methods and treatment conditions for studying effects of fatty acids on cell growth. Lipids 36:305-13
170. **Bronstein I, Fortin J, Stanley PE, Stewart GS, Kricka LJ** 1994 Chemiluminescent and bioluminescent reporter gene assays. Anal Biochem 219:169-81
171. **Giulietti A, Overbergh L, Valckx D, Decallonne B, Bouillon R, Mathieu C** 2001 An overview of real-time quantitative PCR: applications to quantify cytokine gene expression. Methods 25:386-401